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TRANSPLANTATION OF THE CANINE LUNG:  
FACTORS CONCERNED WITH PRESERVATION

A Thesis

Submitted to the Faculty of Graduate Studies  
in Partial Fulfillment of the Requirements  
for the Degree of  
Master of Science (Surgery)

Department of Surgery

By

Richard Terrence Brownlee

Edmonton, Alberta  
September, 1965



UNIVERSITY OF ALBERTA  
FACULTY OF GRADUATE STUDIES

The undersigned certify that they have read and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Transplantation of the Canine Lung: Factors Concerned with Preservation", submitted by Richard Terrence Brownlee in partial fulfillment of the requirements for the degree of Master of Science (Surgery).





## A B S T R A C T

Since Juvenelle in 1951 reported the first successful lung autotransplantation in the dog, numerous investigators have attempted to solve the many problems involved in the transplantation of the pulmonary tissues. Experimental pulmonary function following unilateral orthotopic lung autotransplantation has been shown to be impaired by numerous workers. Functional alterations include diminished graft oxygen uptake, pulmonary hypertension and loss of the Hering-Breuer reflex on the operated side.

When the "homograft reaction" is unmodified lung homografts are rejected in about seven days and function fails early following transplantation. Modification of the immune response results in the prolongation of graft survival in some but not all animals. Ultimately all pulmonary homografts are rejected.

Based on extensive laboratory experience, two human pulmonary homografts were carried out in 1963. In both instances the maintenance of pulmonary viability during the operative ischemic period was a problem.

The problems involved in preservation of the lung for transplantation have not been previously investigated. Therefore the object of this work was to investigate the conditions necessary for the prolonged survival of pulmonary tissue outside the body.

Mongrel dogs were subjected to left lung orthotopic autotransplantation. In the first group the graft was immediately



reimplanted. In the second group the excised lung was cooled to 5 to 10°C for from four to twenty-four hours, then reimplanted.

Pulmonary function studies consisting of arterial pH, pCO<sub>2</sub>, and pO<sub>2</sub>, pulmonary angiography, (a-A)CO<sub>2</sub>, (A-a)O<sub>2</sub>, differential bronchspirometry and right heart catheterization were carried out on all survivors.

In an attempt to extend the limits of preservation by hypothermia alone, dog left lungs were subjected to simultaneous perfusion, ventilation and hypothermia. The effects of autologous blood and homologous plasma, positive and negative pressure ventilation were studied. Parameters measured included: pre and post-perfusion lung weight, perfusate pressure-flow relationships, perfusate pH, pCO<sub>2</sub>, pO<sub>2</sub>, sodium, potassium, chloride, histamine, and proteins. In addition tissue sodium, potassium, chloride, histamine, hydroxyproline, and hexosamine were measured.





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## CHAPTER I

### INTRODUCTION





## T R A N S P L A N T A T I O N

### A B R I E F H I S T O R I C A L R E V I E W

Modern experimental investigation of organ and tissue replacement began in 1862 when Paul Bert studied parabiosis and homograft tolerance in Claude Bernard's laboratory (1). Ullman in 1902 reported "successful" renal homografts and heterografts to a Vienna surgical society (2). Beginning in 1902, Carrel and Guthrie followed up the work of Ullman. In addition to advocating a method of end-to-end vessel suturing still used today (3), Carrel performed many homotransplants (4,5), outlined the surgical techniques of transplanting a wide variety of organs and investigated long-term tissue and organ storage (6). For this work he was awarded the Nobel Prize in 1912.

Despite the great contributions of Carrel and Guthrie to the technical aspects of transplantation, no information was gained from their experiments with regard to the reaction by the host to a homograft because they made no distinction between autografts and homografts. Failure of homografts was attributed to faulty technique, as illustrated by this quotation from Guthrie (7):

"Since I have found no evidence of serious derangement of metabolism in dogs' thighs up to 11 days after transplantation, nor in a dog's foreleg 6 days after transplantation, and since there are no physiologic or other reasons known why such tissues as those found in the limb may not live again



and function under such conditions, it seems justifiable to conclude that it is possible to transplant such a member with permanent success".

In 1923 Williamson, working at the Mayo Clinic questioned the idea that auto and homotransplants behaved alike:

"In the past this failure (of homotransplants) has largely been attributed to external conditions, such as thrombosis of the blood supply, obstruction to the ureter, and infection. But the fact that the autotransplant survives in spite of these factors seems largely to disprove the so-called mechanical theory of failure. It seems to us, that, underlying these failures is some fundamental biological principle which we have not yet been able to identify"(8).

Simonsen, in 1953 described for the first time the hastened rejection of a second kidney from the same donor transplanted into the same host: "The results of these experiments are hardly explicable other than by acquired antibody formation in the recipient against individual-specific antigens in the transplant"(9).

The same year, Billingham, Brent and Medawar published their classic paper on actively acquired immunological tolerance (10). Almost simultaneously, Hume and associates in Boston began the first large series of human kidney transplants (11), and the era of replacement surgery had begun.

#### BASIC PRINCIPLES AND TECHNIQUES.

Definitions: transplantation of living tissue denotes:





- a) removal of tissue from an animal and returning it in a viable state to its original site;
- b) transferring living tissue from one site to another in the same animal;
- c) transferring living tissue from one animal to another;
- d) introducing into, or applying to the animal, viable tissue which has been stored or cultured in vitro (12).

Transplantation may also apply to the transference of non-viable tissue, although a transplant generally implies nutrition by direct vascular anastomosis, whereas an implant consists of thin slices of tissue which ultimately derive their blood supply through regeneration from the host tissue (13). An AUTOTRANSPLANT (autologous transplant) consists of tissues returned to the same individual. An ISOTRANSPLANT (isogeneic transplant) is transferred from one individual to another of genetically identical constitution (identical twins or closely inbred strains). A HOMOTRANSPLANT (allogeneic transplant) implies transference of tissue between individuals with genetically dissimilar constitutions within the same species, and a HETEROTRANSPLANT (xenogeneic transplant) between individuals of different species. Transplants may also be immediate or delayed (as in stored organs or tissues) and ORTHOTOPIC or HETEROTOPIC, i.e. transplanted to their original or a different site respectively.

CONDITIONS NECESSARY FOR AUTOTRANSPLANT SURVIVAL (13)..

As a general rule, once autotransplants become established they survive indefinitely. General conditions necessary for





this establishment include:

1) Initially healthy tissue. Survival of the transplant is unlikely if gross mechanical injury is present or the transplant is stored under unsuitable conditions.

2) Freedom from infection. Under certain circumstances even minimal infection may be fatal to the graft.

3) Adequate nutrition. This factor implies adequate blood supply, supply of nutrients and removal of waste products. With whole organ transplants, blood supply is immediately established by vascular anastomosis (9), but may be impaired or interrupted subsequently by arterial or venous thrombosis.

In the case of whole organ transplants by vascular anastomosis three additional factors obtain:

1) Development of a satisfactory operative technique.

2) Avoidance of irreversible ischemic damage to the organ during the procedure.

3) The capacity of the organ to function in a denervated state. The last factor has been blamed for the inability of renal autotransplants to the neck of dogs to produce urine of high specific gravity (16), and the absence of the Hering-Breuer reflex in autotransplanted lungs (132).

#### THE "HOMOGRAFT REACTION".

An organ transplanted homologously by vascular anastomosis never functions as well as an autotransplant, although their



functions resemble each other approximately for a short time (approximately one week) (14,15,16). The reasons for this disparity in function becomes obvious when one considers the events occurring histologically in a homograft.

Two phases of homograft rejection are apparent (17):

1) An early non-specific reaction characterized by local cellular infiltration (first mononuclear, and later polymorphonuclear).

2) A subsequent and more violent reaction ultimately leading to the destruction of the homotransplant. In the kidney, this reaction is characterized by homograft edema, increasing round cell infiltration, thickening of basement membranes, and changes in intrarenal vessels resembling those seen in periarteritis nodosa. Terminally, there is widespread hemorrhage, edema and necrosis (18).

The foregoing description applies to a homotransplant which is transferred to an individual for the first time and is referred to as the "first set reaction". If a second organ or piece of tissue from the original donor to the same host is transplanted after the first set reaction has occurred, the graft is rejected more quickly and more vigorously (19). This rejection is termed a "second set reaction".

#### HOMOGRAFT IMMUNITY.

It has long been known that tissue grafting could be





performed successfully only between genetically identical individuals. The idea that homograft rejection has an immunologic basis is based on the following evidence (20):

1) The chronology of rejection. The second set rejection is reminiscent of the response of a previously sensitized individual to a second and similar antigenic challenge.

2) Specificity. The second set reaction occurs only against the tissue of the donor supplying the first homograft, or that of a genetically similar individual.

3) Immunologic suppressors. The same treatments which suppress the immune response allow prolongation of the life of homografts in certain instances. These treatments include, total body irradiation (21,22,23,24,25), radiomimetic and antimetabolic drugs (26,27), production of neonatal immune tolerance (29), thymectomy (30) and splenectomy (28,31).

4) Systemic and regional lymph node reaction. Scothorne and McGregor in 1955 (32) showed that nodes draining a skin homograft but not those draining contralateral or draining an autotransplant become enlarged due to increased numbers of large lymphocytes. Generalized lymphocytosis (33,34), and eosinophilia (35) occur in recipients of free homotransplants.

5) Tolerance induction. Fetal animals injected with cells from a potential homograft donor tolerate grafts





from that specific individual in postnatal life.

6) Cell transfer. The homologous immunity state may be transferred to a homograft recipient by means of lymphoid cells (36).

The responsible antigens are probably mitochondrial, granular, microsomal and fibrillar components of cytoplasm (17). At least fourteen histocompatibility loci have been identified in the mouse with eighteen alleles at the H-2, five at the H-1 and an unspecified number of alleles at the H-3 locus (37). The nature of the response to challenge by these antigens has been the subject of intensive research in recent years. Since homograft rejection cannot be transferred passively by serum alone (38,39), the role of antibodies per se in the reaction is questionable. At present a cellular mechanism, similar to that seen in tuberculin or Jones-Mote delayed hypersensitivity reaction seems more likely to be responsible, although a clear explanation of homograft immunity as yet is unavailable (20).

#### TRANSPLANTATION IN SURGERY.

The applications of transplantation, once homograft acceptance and continued function without late sequelae can be guaranteed are enormous to speculate upon. Diseased or irreparably damaged organs could be replaced, malignancy treated more effectively and transplants could be used to



counter the effects of incurable diseases until cures could be found. The transplanted tissues might survive permanently providing complete replacement of the lost tissue, or survive only temporarily, tiding the patient over a crisis.

The surgical technique of replacement is known for a wide variety of organs, including kidney (40), pancreas (41), uterus (42), lung (43), heart (44), and lung and heart combined (45) as well as entire portions of the body such as head and limbs (45).

Autotransplants of skin, bone and cartilage and blood vessels (e.g. arterial vein grafts) are well established as therapeutic procedures. Cervical and/or thoracic esophagus replacement with a right colonic pedicle graft is also performed with moderate frequency. Adrenal autografts have been performed in Cushing's disease (46) as well as kidney autotransplants for high ureteral stricture (47).

Kidney homotransplantation in man has been performed in 672 cases as of December 1964 (48). As an index of homograft failure, only three patients are surviving three years post-transplantation in this group. Lung homotransplants have been performed twice in humans (49,50). One heterotransplantation of the heart has been performed in man. The transplant, however, obtained from a chimpanzee, was too small to maintain the patient's cardiac output beyond six hours post-operatively (51).



Homografts may be obtained from volunteer related or unrelated donors, and as "free" grafts as a consequence of operations involving removal of a normal organ for other reasons or as post-mortem (cadaver) grafts.







## L U N G   T R A N S P L A N T A T I O N

### A.   EXPERIMENTAL AUTOTRANSPLANTATION

#### 1. HISTORICAL REVIEW.

The first efforts directed toward lung transplantation were concerned with only the technical problems. Carrel and Guthrie in 1906 reported results of transplantation of the heart and lungs in combination. Aseptic technique was not used and the animals were not kept more than a few hours. Permanent results are therefore lacking.

"Nevertheless, the circulation was satisfactorily re-established and evidence of return of function was obtained".

Unfortunately, no descriptions of their technique or criteria for return of function are available.

Demikhov in 1946 successfully transplanted homologous heterotopic accessory hearts and lungs into dogs (45). The following year he homotransplanted the right lower lobe in dogs successfully using collodion tube, direct sutured and machine sutured anastomoses. Because of the high incidence of bronchial slough the bronchus was exteriorized or ligated. Seven days appears to be the maximum survival obtained. Whole right lungs were also transplanted but no results of these experiments are cited. Experiments were terminated because of inability to assess the state of the transplant, short of thoracotomy. Demikhov's problem was not unique, for functional assessment of any transplant still remains no easy matter.

Staudacher, Bellinazzo and Pulin in 1950(133), without



previous knowledge of Demikhov's work, evolved essentially the same technique for auto- and homotransplanting the right lower lobe in dogs. The autografts did well for 12 days or more, while homografts were rejected in six to eight days.

In the same year, Lanari, Molins and Croxatto reported results in homografts of the left lower lobe (99), and entire left lung auto- and homografts. They obtained indefinite autograft survival while the homografts were rejected in 7-10 days. This group apparently was the first to report successful orthotopic autotransplantation of the lung.

Also in the same year, Metras (104) homotransplanted the left lung together with a patch of aortic wall in an attempt to preserve the origin of the bronchial arteries. He also was the first investigator to substitute a large atrial cuff for pulmonary venous anastomoses.

Juvenelle et al. in 1951 reported successful results in one animal whose right lung had been orthotopically autotransplanted (134). Thirty five months later the same animal was studied by Portin and associates (114) and participation of the graft in respiration was established. Quantitative results, however, were not obtained. From this stage onward, numerous reports on this subject have appeared, including major contributions by Hardy (43,93,94), Nigro (109,110,111) and Blumenstock and associates (64,68,135).





## 2. TECHNIQUE.

The technical problems of lung transplantation are considerable, as evidenced by the high mortality rates reported by most groups. Alicant and Hardy reported 52% deaths following right or left lung autotransplantation (94), even as late as four weeks post-operatively. Reemtsma's results also indicate 52% mortality (128). Shaw and Burton reported 63% deaths in four weeks (127). Borrie and Lichter reported 100% deaths in 6 days using sheep (70). Blumenstock states that reimplantation was performed on 17 dogs before a chronic survivor was obtained (66). Yeh et al. reported 100% mortality in their initial group with 75% dying in 10 days. This figure was reduced to 24% with systemic heparinization prior to pneumonectomy (125). Similar discouraging results were obtained by Davis et al. who, having evolved the technique in 34 dogs, was only able to complete the procedure in five out of seven dogs, all of whom died within eight days of operation (77).

Although the surgical technique will be described in detail under methods, the procedure consists essentially of the division of the hilar structures, (right or left), and their reconstitution. Only the pulmonary artery, main stem bronchus and venous segments are anastomosed. No attempt is made to restore the continuity of lymphatics, nerves or bronchial vessels. Following the lead of Metras (supra) most investigators have adopted the technique of excising





a cuff of left atrial wall containing the pulmonary veins and anastomosing this either to the original site, or into the left atrial appendage.

### 3. MAJOR COMPLICATIONS AND THEIR PREVENTION.

The above cited early mortality, unrelated to graft rejection, is highly disconcerting when one considers application of lung transplantation to the human. Operative complications, in addition to operative hemorrhage, anesthetic depression etc., are essentially related to:

- 1) The three anastomoses.
- 2) The effects of interruption of blood supply to the lung during the procedure.
- 3) Infection.

#### Hemorrhagic Infarction:

In all series in which complications are enumerated (52,68,70,97,101,103,125,127,128) the primary cause of mortality was venous thrombosis and hemorrhagic necrosis of the lung. Thrombosis may occur at the capillary, venous or atrial suture line level. The atrial cuff anastomosis converts four (on the left) small, difficult venous anastomoses into one large, and relatively easy one, thus enabling better eversion of the anastomotic edges. Eversion decreases contact between blood and traumatized tissues, found to be an important factor in the production of thrombosis (138). The use of dacron sutures causes less reaction than silk in both vascular and bronchial anastomoses (119,138). Prevention



of clotting prior to anastomosis may be accomplished by either systemic heparinization prior to vascular clamping (given intravenously or via the pulmonary outflow tract) (53,89,94,125,127,135), or flushing the graft after excision with a heparinized normal saline (108), or dextran and normal saline solution (70,71,89). The perfusion is continued until the venous return is clear (49,70,78,103,127). Other perfusates include 5% glucose and citrated blood. Most of these agents have been implicated in the production of pulmonary edema (119).

#### Bronchial Anastomosis Complications.

Bronchial suture line disruption, slough, or late post-operative stenosis are considered the second largest group of complications in most series (68,88,104,107,113,119,125,127,137), although some groups have experienced little difficulty in this respect (89,101).

The etiology of these complications is still unclear. Although the presence of the bronchial circulation has been said to be essential for bronchial viability (81), others disagree (102). Stricture can be minimized by denuding the bronchus as little as possible and placing the anastomosis close to the pulmonary hilus (137), as well as obtaining good anastomotic mucosal approximation (74,98). Pneumothorax deaths, secondary to bronchial anastomosis air leakage, have been a problem in some series (68,70,127), but as a rule good mucosal approximation prevents leakage.





The suture line may be reinforced by surrounding tissue (49) or fascia from the aponeurosis of serratus anterior (70).

#### Operative Pulmonary Ischemia:

When one considers the complex anatomy and function of the lung, one is amazed at how much ischemia the lung can tolerate. Normothermic sheep lungs can withstand total ischemia for two and a half hours; longer periods produce pulmonary edema (71). A number of investigators have chilled the lung-flushing perfusate to 4°C in an attempt to induce local graft hypothermia and thus decrease metabolic demands under anoxic conditions (70,78,87,88,128). Reimplantation time, during which the lung is without circulation or ventilation, is variously quoted as being 15 to 120 minutes (52,68,77,85,91). Hardy et al. showed that the lung can regularly withstand two hours of ischemia at 4°C (94). Therefore the lung seems able to withstand an anoxia time at least equal to that required for the operative procedure.

#### 4. PATHOLOGIC CHANGES FOLLOWING LUNG AUTOTRANSPLANTATION.

Early post-operative autopsy material shows marked congestion, bronchial bloody mucus plugs, atelectasis and pneumonitis (126). Lungs from the dogs sacrificed serially following reimplantation however, show only mild congestion, focal atelectasis, and slight eversion of bronchial mucosa with subsequent squamous metaplasia at the anastomotic site (68). Lung expansion is difficult (58,84,89), and interstitial edema of short duration may be seen (58,126). Bronchial





epithelium may be edematous and ecchymotic (58), and ciliary action is probably lost (126). Lymphatic dilatation is seen for approximately five days (97) or may continue indefinitely, in association with increased interstitial fibrosis and obliterated bronchial arteries (111).

Hardy and associates by parenchymal dye injection showed lymph flow in seven days, and within 20 days, dye was seen streaming across the bronchial anastomosis to hilar lymph nodes via regenerated lymphatics (83,93). The period of complete lymphatic stasis correlates well with the period of maximal pulmonary edema (58,97) and functional impairment (see infra).

The alveolar membrane appears normal (49,84,97,108,126) but electron microscopy reveals changes in alveolar macrophages in areas of micro atelectasis (53). Histochemical studies show generalized diminution in enzymatic reactions, suggesting possible impairment of cellular function. It is possible that these alterations might reflect a change in surfactant elaboration following reimplantation (53). Bronchoscopy and bronchography have revealed, in the absence of severe bronchostenosis, little change in the bronchial anastomosis up to three years post-reimplantation (119) and normal or slightly dilated distal bronchial tree with terminal blunting (43,49,68,89).

Aside from these effects of thoracotomy, minimal pneumothorax and atelectasis, chest x-ray may reveal opacification



of the graft between three and eight days post-operatively, again coinciding with the period of maximal edema (83,120,126).

#### 5. PULMONARY FUNCTIONAL ALTERATION FOLLOWING AUTOTRANSPLANTATION.

To determine whether or not the transplanted lung was capable of maintaining life, contralateral pneumonectomy has been performed. The majority of animals died due to inability to re-establish respiratory rhythm (52), or of pulmonary edema due to acute pulmonary hypertension (52,56, 89,108). Physiological limitations of the lung causing death after autotransplantation and contralateral pneumonectomy fall into three categories:

a) Denervation: after extensive dissection around the pulmonary hila and base of the heart (123), autotransplantation of both lungs and heart (124) or bilateral lung reimplantation (52), dogs fail to breath normally and are unable to maintain adequate ventilation. Survival, however, has been reported following total cardiopulmonary transplantation (45, 63,102). Whatever method of pulmonary denervation, the subsequent respiratory pattern is the same; that of slow, deep breathing utilizing accessory inspiratory musculature, a long expiratory pause, and gradual deterioration of breathing until death ensues as long as 36 hours later (52). This type of breathing resembles closely that following cervical vagotomy or thoracic vagotomy and sympathectomy (96).

Contralateral lobectomies have established that at







least 17% normally ventilated and perfused and innervated lung tissue is necessary to ensure a normal respiratory pattern, if the contralateral procedures are carried out shortly after the reimplantation (52). If several months elapse between the initial and contralateral reimplantation or pneumonectomies, survivors can be obtained (79,89,93, 100,116).

Baboons surviving on autotransplanted lungs alone show normal pulmonary artery pressures, arterial pH, CO<sub>2</sub> and oxygen tensions, oxygen uptake and respiratory minute volumes (88). Dogs surviving bilateral lung autotransplantation show normal O<sub>2</sub> consumption and pulmonary artery pressures. Normal alveolar arterial oxygen gradients suggest no significant diffusion or venous shunting defect (100).

The Hering-Breuer reflex from the operated lung is invariably absent, for as long as 35 months following reimplantation (79,114,121,127), indicating an absence of functional vagal afferent fibre regeneration. Portin (114), however, showed nerve fibres distal and proximal to the bronchial anastomosis 35 months following reimplantation, as well as fibres crossing the pulmonary artery suture line, although they were more numerous proximally than distally. No comment was possible as to the degree of nervous regeneration or the function of the fibres seen. Some nerve bundles appeared incompletely filled with nerve fibres.

It therefore appears that some regeneration of innervation



does occur following autotransplantation and that survival can occur after bilateral reimplantation, despite the permanent loss of pulmonary vagal afferent innervation.

b) Pulmonary vascular changes: When the contralateral pulmonary artery is ligated several months following re-implantation, edema develops in the reimplanted lung (52). Both normal pulmonary artery pressures (93,125) and pulmonary hypertension (108,109,110,111), have been observed following autotransplantation. Occlusion of the opposite pulmonary artery (59) or contralateral pneumonectomy results in a further rise in pressure, when pulmonary hypertension is present. Pulmonary hypertension has also been produced by bilateral cervical or thoracic vagotomy (55,56). In no instance has the hypertension been persistent, and it may be absent in dogs following bilateral reimplantation (100). Since left atrial pressures are normal, increased pulmonary vascular resistance at the arteriolar, capillary or venous level must be responsible. There is poor agreement as to whether blood flow is well (128), or poorly maintained through the autotransplant. Tolazoline and acetylcholine decrease pulmonary artery pressure when pulmonary hypertension is present following reimplantation, with a consequent increase in  $O_2$  and  $CO_2$  exchange, inferring a prior vascular spasm with decreased blood flow (109).

This increased pulmonary vascular resistance has been linked with the lung autograft's susceptibility to pulmonary





edema. Hardy et al. (93) divided the hilar structures differentially and evaluated subsequent pulmonary function. Division of bronchial arteries, lymphatics and nerves about the bronchus resulted in impaired oxygen uptake and ventilation with a high incidence of pulmonary edema and hypertension. Bronchial artery division alone produced few functional changes. Bogardus (69) reported contrary results; bronchial artery division precluded survival following contralateral pneumonectomy due to edema and hemorrhage into the remaining lung. The pulmonary artery and veins can be divided and reanastomosed in sheep with no pulmonary functional impairment (70).

c) Ventilation-perfusion relationship changes: Arterial blood gases tensions may be normal (73) or oxygen tension or saturation may be decreased (125) following unilateral autotransplantation. Normal blood gases in the presence of a patent pulmonary vasculature, as demonstrated by angiography, determine only that the transplant is participating in respiration. The remaining studies are designed to determine how well the transplanted lung carries out this participation.

Following reimplantation the respiratory quotient is unchanged and the ventilatory equivalent rises. The alveolar arterial oxygen gradient and venous admixture increases (125). Oxygen uptake by the transplanted lung, as assessed by differential bronchspirometry, falls abruptly following surgery, to about one-half control levels, then gradually increases over two weeks toward normal levels. In some





instances this return of function never reaches control values despite normal ventilation (128), indicating perhaps, inadequate perfusion (137), or a diffusion defect (128).

Reemtsma and associates report normal blood flow through autotransplanted lungs but report no method or results (128). Functional separation of the right and left bronchi, upon which these differential studies depend, is difficult in the dog (118,128), and in the regions where the majority of these studies were carried out, heartworms are endemic (93,112,128), undoubtedly influencing results. Slim et al., studying dogs with bilateral reimplanted lungs showed a significant alveolar-arterial oxygen gradient when breathing 100% oxygen, but did not differentiate between perfusion and diffusion defects (116). Their results, however, indicate a perfusion defect in association with increased pulmonary vascular resistance and stenosis of the pulmonary artery anastomosis.

Lempert and Blumenstock under the same conditions reported no significant diffusion defect or venous-arterial shunting (100). Here again, results are contradicting, pointing out the difficulty in assessment of ventilation-perfusion relationships in the dog (116,125).

Pulmonary compliance is decreased (108,109,116) in the face of normal surface tension measurements following autografting (121).



### B. EXPERIMENTAL PULMONARY HOMOTRANSPLANTATION

#### 1. UNMODIFIED HOMOTRANSPLANTS.

Lung homotransplants follow essentially the same course as other organ transplants when the reaction is unmodified by treatment. The animals appear healthy, eat and walk on a leash for approximately two to three days. The lung is aerated and functioning (57). Thereafter, the animal becomes listless, refuses to eat and dies in approximately six days. Grossly the transplanted lung is edematous, dark red and solid. Histology shows consolidation and alveolar necrosis (54), with congestion, thrombosis and infiltration (77). Angiograms show patency of major vessels but failure of filling of smaller branches of the pulmonary artery (120). It is clear that since some vessels become dilated and engorged and then undergo thrombosis and necrosis, that ischemia probably contributes to the ultimate demise of the homograft (17), although vascular thrombosis may not be noted, and even after the donor lung has been encapsulated by the host and has lost most of its architecture, a normal angiogram may be obtained (72).

Homografted lungs show the same immediate impairment of oxygen uptake as autografts but the autograft's functional return toward normal is absent. Function actually diminishes progressively. Reemtsma reports initially normal blood flow through homografts, but again no method, data or later follow-ups are reported. Function has ceased completely in left lower lobe transplants by 72 hours, as assessed by oxygen







uptake, histology and histochemistry. Tissue water  $\text{Na}^+$  and  $\text{Cl}^-$  content increase after 24 hours. Cessation of function is attributed to interstitial edema, and perivascular and peribronchial cell infiltration (87).

Homograft and autograft compliances are initially similar, but 48 hours post-transplant, homograft compliance and surfactant are markedly reduced, indicating alveolar cell damage. Since the homograft appears relatively normal grossly and microscopically at this time, and since a 24 hour lag ensues between cessation of surfactant production and surface tension alteration, changes in surfactant and compliance may be the earliest detectable signs of homograft rejection in the lung(121).

In summary, most lung homotransplants are rejected resulting in recipient death within approximately one week in the absence of immunosuppressive therapy. Occasionally, however, homotransplants may survive for prolonged periods with residual function (72,115), or may be rejected without host death (43,57,70,97). Functional impairment begins quickly, is progressive and can be correlated with the histologic and biochemical events occurring during the homograft reaction.

## 2. MODIFICATION OF LUNG HOMOGRAFT REJECTION.

In theory, elimination of the homograft reaction could be produced by blocking either antigenic release from the transplant, blocking the reaction of the host to these antigens, or by the induction of specific immune tolerance.



Although it is generally agreed that constituents of the intracellular organelles are the major contributors of antigens responsible for homograft rejection (17), considerable controversy still exists as to whether the responsible antigens are common to all tissues or can at times be organ-specific. Common antigenicity between skin and lung homografts exists, since following skin grafts between the same individuals, are rejected in a second set fashion (140). Quick freezing to  $-79^{\circ}\text{C}$  leaves lung antigenicity unaltered. Graft irradiation prior to transplantation might alter its antigenicity, but to our knowledge this approach has never been attempted.

Attempts to modify host reaction with antihistamines or total body irradiation, with or without bone marrow transplants, have been unsuccessful (64,65,66,68,91,107). ACTH and adrenal corticosteroids alone increased survival time slightly (92), however, these results could be unrelated to the immune response (see discussion). Cytotoxic drug therapy has been more successful. Post-transplant treatment with methotrexate, a folic acid analog, increases survival (64,65,66,68), however, pulmonary function is still eventually lost (43,64). Host tolerance, seen in certain humans receiving homologous kidneys does not develop and the drug therapy, as a rule, cannot be discontinued (64,65,66,68). Azathioprine therapy approximately quadruples mean survival time, and little difference is noted when adrenal steroids are added to the regimen (43). Other workers have noted diminution or absence





of homograft reaction in only one-half homograft animals treated with this drug (57).

The symptoms of methotrexate toxicity are related to the hemorrhagic desquamating enteritis and bone marrow depression which can occur (65). Azathioprine may cause cholestatic jaundice (95). By comparison, azathioprine produces less toxicity and better immunosuppression than methotrexate (113). Other drugs improving homograft results include cyclophosphamide (103) and actinomycin-C (93,94,103).

In summary, the behavior of lung homotransplants differs greatly from that of autotransplants because of the immune mechanisms involved. If rejection is unmodified, there is an immediate impairment of function which progresses relentlessly and results in the early demise of the host. Even the best immunosuppressive therapy produces fair to good results in only one-half the animals subjected to homografting, and in these animals chronic rejection still occurs. In addition to their inability to produce prolonged lung homograft tolerance, presently available immunosuppressive drugs are non-specific and produce highly undesirable side effects, which on occasion can kill the host. For these reasons, lung homografts are unsuitable for the study of in vitro organ preservation.

#### C. LUNG HOMOTRANSPLANTATION IN HUMANS

"Twice in the last 15 years there have been surprisingly false predictions from experts in biological fields. In the





early 1950's a satisfactory extracorporeal pump oxygenator was regarded as an impossibility; homotransplantation of tissues in man was regarded merely as a visionary dream. In this short span of time, both have been shown to be possible".

Francis D. Moore, 1964.

Despite the attractiveness of transplantation as therapy in conditions in which the lungs have been damaged extensively by trauma, degenerative, neoplastic or cardiac disease, surgeons throughout the world with extensive laboratory experience in this field have been cautious in applying this experience clinically. The major problems of functional alteration following even autotransplantation and the present inadequacy of immunosuppressive therapy are the main factors creating this reticence.

Dr. Hardy, in 1963, gave considerable warning that he intended to do a human lung homotransplant. In discussing his laboratory studies and their clinical potential, he said:

"Clinical homotransplantation of the lung should prove essentially as feasible as homotransplantation of the kidney. In fact, in acute self-limited conditions such as massive and otherwise fatal pulmonary aspiration, or even specific pneumonitis, the temporary insertion of a lung homograft could represent the only means of gaining enough time for the contralateral lung to regain functional integrity. At present the cautious experimental management of terminal



chronic pulmonary insufficiency or cor pulmonale by lung homotransplantation could be justified only in otherwise hopeless cases"(94).

Thus, on June 11th, 1963, at the University Medical Center, Jackson, Mississippi, Dr. Hardy and associates performed the first human lung homotransplant.

The patient was suffering from an occlusive carcinoma of the left mainstem bronchus, with complete atelectasis of the abscessed left lung. There was an associated empyema. Complicating factors included severe functional limitation of the right lung due to emphysema, malnutrition, dependent edema and moderately far advanced chronic renal disease.

After carefully considering the technical feasibility and moral justification for the operation it was decided to proceed. A potential donor entered the emergency department in shock with pulmonary edema secondary to a massive myocardial infarction. Efforts to resuscitate him failed and permission for autopsy was obtained from the relatives. Ventilation with pure oxygen via an endotracheal tube and external cardiac massage were maintained while the patient was moved to the operating room. Heparin was injected into the heart immediately after death. The donor's left lung was removed, the main stem bronchus cannulated with a sterile tube and rhythmic inflation with pure oxygen instituted and continued until the vascular anastomosis could be completed.

Meanwhile, the recipient's lung was removed in an adjacent







theatre. Because of larger structures in the human than the dog, it was found that pulmonary venous anastomosis would be satisfactory. These were completed first. The pulmonary artery was next anastomosed; the sterile tube was removed from the bronchus, and that anastomosis was carried out. At this point in the procedure, Dr. Hardy and his associates must have had a bad moment:

"The operators were somewhat dismayed then, when it appeared that it was going to be difficult to get the donor lung into the left hemithorax and close the chest, but it gradually developed that this could be achieved satisfactorily" (143).

The films of the procedure, shown subsequently by Dr. Hardy confirmed this point (130).

The pulmonary edema, seen in the donor lung initially, gradually subsided with positive pressure ventilation. Thymectomy and splenectomy were decided against, and the chest was closed. The operation lasted three hours, and the donor lung was without circulation for 90 minutes (2 hours from the actual donor death).

Pre-operatively arterial  $O_2$  saturation was 87.3%. Immediately following clamp removal it rose to 98.58%. Seven days later it was 94.18% and remained unchanged until death ensued. Operative vascular pressures were: aorta, 145/80; left pulmonary artery 35/20 and inferior pulmonary vein 20/15 mm.Hg. The increased venous pressure was thought to



be due to the increase in vessel size from recipient to donor. The patient emerged promptly from his anesthesia, appearing to have tolerated the procedure well. Intravenous low molecular weight dextran was instituted immediately, and 24 hours later heparinization was commenced to prevent graft vascular thrombosis. Angiocardiography on the first day demonstrated graft perfusion. Immunosuppression was achieved with azathioprine and mediastinal and thymic x-irradiation.

Despite excellent pulmonary function, the patient died on the eighteenth post-operative day of renal failure, residual tumor and general debility. Autopsy revealed little difference between the two lungs. Vascular and bronchial patency were demonstrated, compliance of the two lungs was grossly equal, and microscopic studies revealed well preserved alveolar architecture, with no evidence of either infection or rejection.

Almost simultaneously, in July 1963, Magovern and Yates at the Presbyterian University Hospital, Pittsburgh, Pa., also decided that they were ready to perform their first human lung homotransplant. Their indication for the procedure was:

"Each team must make its own decision as to when it is prepared to approach the clinical application. Our decision was dictated by having an otherwise healthy patient with advanced emphysema, a suitable 21 year old healthy donor dying of a ruptured cerebral aneurysm, an enlightened host





family, and a considerable experience in canine lung transplantation prior to clinical application"(50).

The potential donor, a patient on the neurosurgery service, morally and legally died 10 days following a craniotomy. Immediately, an external cardiac pacemaker was utilized in an attempt to gain some lung perfusion and ventilation was continued with a positive pressure respirator. Generalized body hypothermia was already in use for treatment of his neurosurgical condition. After systemic heparinization, the donor's left lung was removed and cooled in sterile Ringer's solution. The recipient's left lung was removed and the anastomoses performed; venous first, then pulmonary artery and bronchus. Following completion of the arterial anastomosis perfusion was restored. Total graft anoxia time was 90 minutes. The bronchial anastomosis was reinforced with Teflon felt. Although he deteriorated somewhat following the removal of his own lung, the patient tolerated the procedure well. The following day he again deteriorated and convulsive episodes ensued. It was postulated that his  $\text{PaCO}_2$  had been lowered so quickly that a state of central nervous system alkalosis had been created. After cessation of positive pressure ventilation, he regained consciousness. Later, while on assisted (versus controlled) respiration, his arterial pH was 7.38 and his  $\text{PaCO}_2$  55 mm.Hg.

Immunosuppressive therapy was confined to 4-amino- $\text{N}^{10}$ -methylpteroylglutamic acid (methotrexate) and was continued





from immediately post-operatively until death.

Bacterial cultures at operation from the donor bronchial stump grew coagulase positive staphylococcus and pseudomonas organisms. Massive antibiotic therapy was instituted, but on the fifth post-operative day the patient began to deteriorate and died three days later.

At autopsy there was gross evidence of infection of both the graft and left pleural space, and/or graft rejection. The anastomoses were intact, with no evidence of thrombosis. Microscopic examination of the graft revealed intense, necrotic alveolar walls. After intensive investigation including bone marrow, spleen and lymph node examination, and fluorescent antibody studies of both lungs it was concluded "that changes in the transplanted lung could be due to infection alone or to a combination of infection, and an immune rejection reaction"(50).

Demonstration of the possibility of pulmonary homografting in man represents perhaps the most striking advance in thoracic surgery in the last few years. In both cases, due to the rigid moral criteria for operation set up by the surgeons, the procedure was carried out in desperate circumstances. Perhaps in the future better results may be obtained by giving the patient a graft before this state of affairs is reached. A moribund patient already has poor defenses against infection; immunosuppressive therapy, could, under these conditions, eliminate the remainder. Any existing



infection in the donor lung might then result in the rapid demise of both graft and recipient. In the lung transplant, this problem becomes doubly important, and: "one wonders, in a lung transplant, which is almost impossible to separate from contaminated air, that until a specific agent is developed for controlling the response without suppressing resistance, if infection will not always be more of a problem than it already is in liver or kidney transplants"(50).

Both cases were managed without technical incident, pointing out the greater ease of transplantation in the human, as opposed to the dog. All anastomoses were larger and anastomotic stenosis less likely. Excision of an atrial cuff for the venous anastomosis is not necessary for the same reasons.

Hardy's problem with donor-recipient size disparity could have been avoided pre-operatively, since it is immediately apparent that the lung, like the brain, is tightly enclosed in a rigid structure. The human lung, like that of the experimental animal seems to tolerate the operative ischemia well under the imposed conditions. Similarly, the diseased contralateral lung did not prove to be a major problem. If difficulties did arise, short term partial or total cardio-pulmonary bypass could have been employed.

In the patient's post-operative course, it becomes apparent that little of the functional impairment seen following transplantation in the dog is seen in the human.







There was no increase in pulmonary vascular resistance, no decreased compliance, nor significant alveolar-arterial gas gradient attributable solely to the homograft (implying good perfusion, diffusion and ventilation). Pneumothorax was not a problem in either case. Graft vascular thrombosis is less likely to occur in humans than in animals, due to larger anastomoses and better mucosal approximation. When one considers that the experimental animal has a normal contralateral lung, the dichotomy between experimental and clinical results becomes all the more striking. In fact, Magovern's major early post-operative complication (convulsions) was caused by the graft functioning too well, rather than not well enough.

Immunosuppressive therapy in Hardy's patient was certainly successful, and may have been successful in Magovern and Yates recipient. However, since Hardy's donor and recipient were subsequently shown to share eight of thirteen blood groups tested, one wonders whether this high degree of accidental histocompatibility was not more important to the survival of the graft than the suppression therapy.

Utilization of a living lung donor is, and probably always will be, out of the question, in view of the high degree of functional impairment pneumonectomy produces. Thus, future investigators will have to rely once again on cadaver donors. The problem of treatment of the donor while the potential graft is still in the body, and of the excised graft prior to transplantation again comes to the fore.



## O R G A N   P R E S E R V A T I O N

### 1. IMPORTANCE IN TRANSPLANTATION.

Because long term whole organ preservation is at present impossible, one of the major problems in clinical organ transplantation is to have a suitable donor organ available at precisely the time it is needed. With transplantation of unpaired organs and certain paired organs it is necessary to use cadaver donors. However, potential cadaver organ donors do not always die at convenient times, and a suitable recipient is often unavailable at that precise moment. Thus, although the demand for organ transplants far outstrips their supply, suitable donor organs may be lost through lack of ability to maintain graft viability in vivo following donor death. At present, only empirical methods of short term organ preservation, which are expensive, elaborate and subject to technical failure are available. In addition, little is known about the tissue alterations involved in cell death or following preservation procedures. With the current enormous advances being made in the clinical application of the surgery of replacement and immunosuppression, and although orthostatic graft preservation methods are well established and artificial internal organs on their way, surgeons may be "caught short" in their knowledge of conditions necessary for long term organ preservation unless considerable new information in this field is forthcoming.





## 2. AVAILABLE METHODS OF STORAGE AND PRESERVATION.

Since whole organ culture by means of in vitro perfusion was first investigated by Carrel and Lindbergh, many of the problems defeating early investigators have been solved. Infection can be controlled by antibiotics, blood clotting prevented by anticoagulants, and with the advent of the extracorporeal pump-oxygenator, a well oxygenated pulsatile blood flow can be maintained for long periods (141). The mechanism of blood trauma in extracorporeal circuits has been extensively investigated and synthetic media for organ perfusion have been developed.

In view of these advances, one would think that the prolonged maintenance of isolated organ viability would become easy, but in the words of Ian Aird, with respect to isolated organ perfusion, "after some hours the perfused organ swells visibly and the outflow of blood from it progressively diminishes". The reason for this failure is unknown (155).

Of the current methods available for tissue preservation, most are not applicable to the problem of whole organ storage. Tissue culture methods depend upon diffusion of nutrients into the tissue in question; if the tissue mass is too great, central necrosis occurs (142). Tissue preservation by freezing is not generally successful, due to cell damage and death caused by mechanical disruption of cellular constituents (143,144,145) and electrolyte or solute concentration (146, 147,148) during intracellular ice crystal formation. In





addition, an injury termed "thermal shock" may result from sudden temperature change, attributed by Lovelock to thermal expansion (149). Factors determining the fate of frozen cells include cell permeability, the rate of cooling and rewarming and the conditions and duration of storage (150). Treatment with glycerol and/or dimethyl sulfoxide prior to or during freezing prevents many of these alterations. Rat skin grafts treated with 10% dimethyl sulfoxide prior to freezing to  $-79^{\circ}\text{C}$  for short periods survived in 95% of the cases. The addition of hyperbaric oxygenation to the above regimen gives additional protection (151).

Dog livers subjected to 15% vacuum dehydration, immersion in dimethyl sulfoxide or glycerol with added glucose and albumen were stored for up to five days at  $-6^{\circ}\text{C}$ . Following homotransplantation into the pelvis or groin of a dog, bile production and substantial, although reduced enzymatic activity occurred until rejection supervened (152). Hamster hearts show no in vitro impairment in function following freezing to  $-2^{\circ}\text{C}$  for thirty minutes; when gradual cooling with glycerol perfusate was used, resumption of beating occurred after freezing to  $-20^{\circ}\text{C}$  in some hearts (153).

Fixation of grafts by formalin, alcohol, merthiolate or heat causes gross alteration in protein structure and cell death (154).

#### Hypothermia without freezing:

Temperature reduction finds application in organ preserv-



ation due to the striking way in which it lowers metabolic rate. Generally speaking, oxygen uptake falls with diminution in temperature, both with respect to the intact organism and isolated organs (156-161). Smith said: "The basic principle in storing living cells is to arrest the processes of ageing and degeneration. Cooling causes a slowing down of the biochemical processes involved in respiration, metabolism and all the other interactions between the cytoplasm of the cells and their environment" (162). When the temperature is  $28^{\circ}\text{C}$ , oxygen consumption falls to 50% of normal; when  $20^{\circ}\text{C}$ , 20% of normal (159). Near freezing, oxygen consumption is too low for measurement (156). Levy in 1959 showed that between  $5^{\circ}\text{C}$  and  $15^{\circ}\text{C}$ , oxygen consumption, and thus metabolism, are minimal (163). Protection of the ischemic kidney by hypothermia has been demonstrated on numerous occasions (164-167). Hypothermia may be accomplished by surface cooling (168-170), or by vascular perfusion (171,172).

Using surface cooling in order to obtain a rapid fall in organ core temperature, it is necessary to use very low temperatures in contact with the organ surface, causing surface necrosis and delayed healing (172,173).

Using perfusion cooling, the organ core temperature rapidly assumes that of the perfusate (173). When blood is used to perfuse the kidney, increased perfusion pressure is necessary to maintain the flow below  $26^{\circ}\text{C}$  (171,173,174). This effect has been attributed to increased vascular resist-







ance (156,173), and the physical properties of blood (173). When plasma, serum, or a blood-dextran-saline mixture is substituted for whole blood as perfusate, the phenomenon is not seen (173,175,176). At low temperatures, sludging of red blood cells and plasma escape into tissues occurs (156, 173), with possible blocking of the fine peripheral vessels in the absence of vasoconstriction (175,176). Probably for the same reasons, hemoconcentration occurs in total body hypothermia (178). Increased cerebrovascular resistance during hypothermic brain perfusion can be alleviated by CO<sub>2</sub> inhalation and arfonad (177). The hemoglobin dissociation curve for O<sub>2</sub> shifts to the left, resulting in decreased oxygen availability (179) which can be increased somewhat by hemodilution (180) and hypercapnia (181). Blood P<sub>O<sub>2</sub></sub> and P<sub>CO<sub>2</sub></sub> and pH vary markedly with temperature (182).

#### Hypothermic protection of the ischemic lung:

Blades et al., produced normothermic lung ischemia by ligating the left hilar structures in situ. Temporary occlusion of all hilar structures under these conditions resulted in surprisingly little gross or microscopic change in the lung following release of the ligatures, with anoxia times of up to 360 minutes (61). Subsequent work, however, showed that hilar occlusion times of greater than 30 minutes increased susceptibility to pneumonia, and two thirds of the dogs subjected to 90 minutes occlusion died. Unfortunately, no data is presented as to the cause of death in this



group. Thirty minutes of occlusion was suggested as the upper limit for survival of function (62). It should be noted that these lungs had innervation, bronchial circulation and lymphatics intact following ischemia, an advantage not shared by the autotransplanted lung.

When the pulmonary artery is occluded for three hours in situ (hence at normothermia), the compliance of the ischemic lung is markedly diminished two days later. At the same time, surfactant is decreased by one-third (183). Again, assessment of this information in relation to lung transplantation is difficult, since under these conditions, the lungs would remain ventilated and subject to retrograde venous pressure during the period of pulmonary artery occlusion.

Borrie and Lichter, using sheep, divided the bronchial artery and occluded all hilar structures individually. They concluded that the left sheep lung will regularly withstand two and one half hours of anoxia, that there is no sharp end-point to the lung's tolerance to anoxia, and that after four hours of hilar normothermic occlusion pulmonary edema will regularly occur (70).

Hardy et al. utilized hypothermia in attempts to maintain lung viability for prolonged periods prior to re-implantation. The lung vasculature was flushed with lactated Ringer's solution, low molecular weight dextran, heparinized saline or 5% dextrose. The flushing solution was then replaced with Tyrodes solution and the lungs were stored at





4°C for varying periods of time and reimplantation was performed. Lactated Ringer's solution, low molecular weight dextran and heparinized saline as flushing solutions precluded survival. Seven out of 42 dogs survived; two after lung storage of 24 hours, 3 after 2 hours, 2 after one hour. Dextran was administered prior to operation, but apparently no anticoagulants were used. Unfortunately, no data regarding specific numbers of animals in each group, total lung anoxia times, or post mortem findings are presented. One animal surviving reimplantation of a lung stored for 24 hours had an oxygen uptake of 50% of the control value for the operated lung.

Pulmonary artery pressures rose immediately post-operatively and remained high for a period which is not mentioned. Survivors had almost normal lungs grossly and microscopically at sacrifice at an again unmentioned time post-operatively (93).

Blumenstock and associates evaluated hypothermia in combination with various substances replacing the pulmonary blood during lung preservation for 18 to 28 hours (67). After removal of the lung from the donor, the left pulmonary artery was perfused for a short period with serum or dextran. The graft was then placed in a plastic bag, immersed in ice water and ventilated with 100 ml. of air three to four times a minute. Following the preservation procedure, orthotopic homotransplantation was carried out. All animals developed





post-operative pulmonary edema. No replacement of the blood in the pulmonary vasculature, replacement with macromolecular weight dextran, serum or serum and dextran all resulted in lung necrosis or complete graft fibrosis. When replacement was made with buffered serum treated to remove all CO<sub>2</sub>, four of six animals survived, showing lung histology similar to that seen in methotrexate treated homografts not subjected to preservation. Unfortunately, no pulmonary function studies or lung parenchymal temperatures are reported on these animals. In addition, post-operative assessment is made difficult by the super-imposition of rejection phenomena.

### 3. ORGAN PRESERVATION BY PERFUSION.

Some of the problems involved in hypothermic and normothermic organ perfusion have already been mentioned. Carrel and Lindberg, between 1914 and 1938, were able to preserve small organs (e.g. ovaries) for as long as 28 days utilizing a special organ perfusion chamber and a variety of perfusates. Their techniques were, however, rather inadequate by modern standards and no attempt at evaluation of graft viability was made aside from histologic appearance. They were unable to preserve large parenchymal organs using the same technique (6). The majority of more recent work has been concerned with kidney preservation. The kidney appears to tolerate profound hypothermia or freezing for prolonged periods better than continuous vascular perfusion. Lapchinski in 1960 reported survival of sufficient renal function follow-



ing 28 hours of storage at 4°C to maintain life in some dogs. Perfusion with cold blood was utilized only at the beginning and the end of the storage period (184). Night et al., evaluating function of renal autografts following six hours of storage, found that neither perfusion or perfusates composition affected graft survival or function. The increased rate of cooling when hypothermic perfusion was used, however, seemed to confer better organ protection than surface cooling (185).

Markland (198) in 1964 evaluated various perfusates including dextran-saline (normothermic and hypothermic); normothermic serum; heparinized autologous blood; heparinized dextran, papaverine and blood; dextran, papaverine, heparin and blood; and hypothermic dextran and THAM, in attempts to maintain viability in in situ rabbit kidneys. Tissue survival was graded I to V depending on the severity of functional and microscopic alterations. The best perfusate was found to be 6% dextran in saline with added heparin and papaverine.

Couch et al., succeeded in maintaining urine production in perfused kidneys for up to 7 hours, but subsequent auto-transplantation was unsuccessful (186,187).

The main difficulty encountered in in vitro kidney perfusion is progressive vasoconstriction and edema. Telander was able to eliminate this problem with dextran hemodilution with normothermia (199). Rosenfeld and associates observed increased isolated kidney blood flow when all glass and





stainless steel surfaces were siliconized (200).

To our knowledge, in no experiment involving isolated lung perfusion has vasoconstriction been a problem. Since all kidney transplant perfusion experiments have been directed toward elimination of vasoconstriction or decreased renal blood flow, it is difficult to evaluate the relationship of this work to perfusion lung preservation.

Pulmonary perfusion has been performed in the investigation of the lung as a biologic oxygenator for cardiopulmonary bypass, normal lung physiology and the chemotherapy of malignancies.

Verney and Starling (188) used a heart-lung preparation to perfuse isolated kidneys. Hemingway showed that the lung would eliminate vasoconstrictor activity present in defibrinated blood, as well as oxygenate the perfusate (189).

Wesolowski et al., attempted to perfuse dog lungs with human blood, but severe congestion and pulmonary edema occurred after 20 minutes (196).

Subsequently, Crisp, Campbell and Brown successfully used dog lungs as oxygenators in human extracorporeal circulation. They attributed their success to:

1. Careful perfusion of the dog lung with dextran at low pressures to remove all dog blood.
2. Depulsation of the pulmonary arterial pressures.
3. Unobstructed pulmonary veins.
4. Lung ventilation with pressures of no more than 16 to 20 cm. of H<sub>2</sub>O.



5. Avoidance of expiratory obstruction.

6. Heparinization of the donor dog.

Under these conditions the lungs would tolerate flows of up to 3000 cc./min. at pressures of less than 25 cm. of H<sub>2</sub>O. Although the presence of edema in the dog lung was denied, published photomicrographs show considerable interstitial edema (190,191). Mustard and Chute used primate lungs as oxygenators in operations involving extracorporeal circulation in children (197).

Isolated canine lung perfusion has been investigated widely in connection with chemotherapy of pulmonary neoplasms (201-204). As a general rule, perfusion time was limited to ten to twenty minutes, too short a period to allow any great degree of correlation with preservation procedures. The addition of highly toxic drugs to the perfusate also complicated matters, however a few pertinent observations were made. Pierpont states that only whole blood should be used as a perfusate, as plasma or balanced electrolyte solutions produce a "wet lung". Perfusion rate should not exceed 175 ml./min. which, under the conditions of their experiment maintained a pulmonary artery pressure of 40 mm.Hg. Rhythmic inflation of the lung was necessary to permit even dispersion of the perfusate (201).

Physiologic knowledge of the behavior of the pulmonary circulation, and the effects of inflation of the lung on its hemodynamics has increased rapidly since 1954 when Riley





and Cournand published their classic papers on pulmonary ventilation-perfusion ( $\dot{V}_a/\dot{Q}$ ) relationship (192).

Other key contributors to the understanding of the behavior of the isolated perfused lung have been Duke, on the regulation of pulmonary blood flow (194), Daly, concerning pulmonary vascular reflexes (210), and Bannister, Torrance and Permutt et al., on the "sluice hypothesis" of pulmonary blood flow (195,205).

From these and other studies the idea of the uniqueness of the pulmonary, as compared to the systemic circulation, has evolved.

Anatomically, the pulmonary vessels are extremely thin walled (193). Hence their hemodynamics can be influenced markedly by passive effects imposed upon them by external events, such as intrathoracic, alveolar, and left atrial pressure changes. They also lack arterioles (193), converting the system into a low resistance circuit with a high blood volume capacity (194,206). In addition, intravascular pressures are one fifth those encountered in the systemic circulation. Blood flow in pulmonary capillaries is pulsatile (207), and numerous arteriovenous shunts at approximately the 20 micron level have been demonstrated (194,208,209,210). Active vasomotion is controlled by alveolar and blood gas partial pressures, hormonal and vagal influences (194,211).

Although isolated perfused lungs have been used on numerous occasions in the elucidation of these mechanisms,



to our knowledge, the lung has never been perfused and ventilated for the purposes of transplantation preservation, although one recent study had as its object the prolonged maintenance of viability in in situ isolated perfused pulmonary lobes in the dog (212).

#### 4. PRESERVATION WITH HYPERBARIC OXYGENATION (HPO).

a) Principles: (213): Under normal conditions the  $P_{aO_2}$  is about 100 to 130 mm.Hg. Pure oxygen administered via a mask results in a  $P_{aO_2}$  approximating atmospheric pressure (about 670 mmHg.). Increasing the ambient pressure in a pure  $O_2$  environment to 3 atmospheres absolute results in a  $P_{aO_2}$  of approximately 2000 mm.Hg. All of the increase in  $P_{aO_2}$  with increased pressure is the result of increased dissolution of oxygen in plasma. Under these conditions, one could in theory, overcome virtually any defect in oxygen transport.

At three atmospheres  $O_2$ , only one half normal perfusion is necessary to oxygenate most tissues. Increased tolerance to complete ischemia at ambient pressures also occurs, if the tissue has been previously hyperoxygenated. Hypothermia increases the solubility of oxygen, and slows its extraction by the tissues. At three atmospheres absolute, and temperatures approaching  $0^{\circ}C$ , one in theory could anticipate 0.6 cm.  $O_2$  diffusion through solid tissue (215). HPO has also been shown to decrease the production of lactic acid,





a product of anaerobic glycolysis, by anoxic tissues (214).

Countering these obvious advantages of HPO in organ preservation are, unfortunately, numerous disadvantages. Because hemoglobin under HPO conditions never becomes reduced, CO<sub>2</sub> transport can markedly be impaired, resulting in a respiratory acidosis at the tissue level. Although red blood cells seem almost superfluous under these conditions, the decreased blood viscosity gained by hemodilution or synthetic perfusates is countered by the direct and/or indirect vasoconstriction produced by oxygen under pressure.

HPO per se appears to be toxic to certain tissues, most notably the lung and the brain. Pulmonary damage is in the form of atelectasis, hyperemia and edema in the intact animal (217,218). Animals surviving these acute changes show subsequently pulmonary arteriosclerosis (219). There is a suggestion, however, that at least the acute changes are secondary only to atelectasis and can be completely reversed with forceable positive pressure inflation (220).

b) Application: Manax, Lillehei and associates have applied these principles to the preservation of kidneys, lungs and hearts transplantation. Under the imposed conditions, kidneys could be successfully preserved for 24 hours at 2°C and 3 atmospheres OHP, or for 48 hours at 2°C and 7.9 atmospheres OHP (216). The criteria for success was the ability



of a single autotransplanted and preserved cervical kidney to sustain life following contralateral nephrectomy. Under similar conditions dog lungs survived storage for 48 hours at 2°C and 3 atmospheres OHP (215).





## CHAPTER II

### METHODS AND APPARATUS



## 1. IMMEDIATE AUTOTRANSPLANTATION.

a) Surgical Technique: Forty-one mongrel dogs weighing 12 to 35 kgs. were subjected to autologous orthotopic transplantation of the left lung. With minor modifications, the method of Hardy et al. (52) was used. All dogs were anesthetized with pentobarbital sodium, 30 mgm./kg. intravenously. If supplementation during the procedure was required, 10 mgm. Atravet was given intravenously. All animals were placed in the right decubitus position. An endotracheal tube was inserted, the cuff inflated and controlled positive pressure respiration with an air-97% O<sub>2</sub> mixture instituted, with a pressure of 10-15 cm. H<sub>2</sub>O and a ventilatory rate of 20/minute. Pre-operative arterial blood samples for PaO<sub>2</sub>, PaCO<sub>2</sub> and pH were taken by sterile percutaneous femoral artery puncture, either at this time or prior to the institution of controlled ventilation. The previously shaved left hemithorax was prepared with betadine and draped. An intravenous infusion of 5% dextrose in water was started and continued until near the end of the procedure. Under aseptic conditions a left posterior thoracotomy in the fifth intercostal space was made, dividing latissimus dorsi, serratus anterior and the intercostal muscles (Fig. 1). After rib retraction, the left inferior pulmonary ligament was divided (Fig. 2) and the pericardium incised inferior to the pulmonary hilus and superior to the phrenic nerve. The dissection was





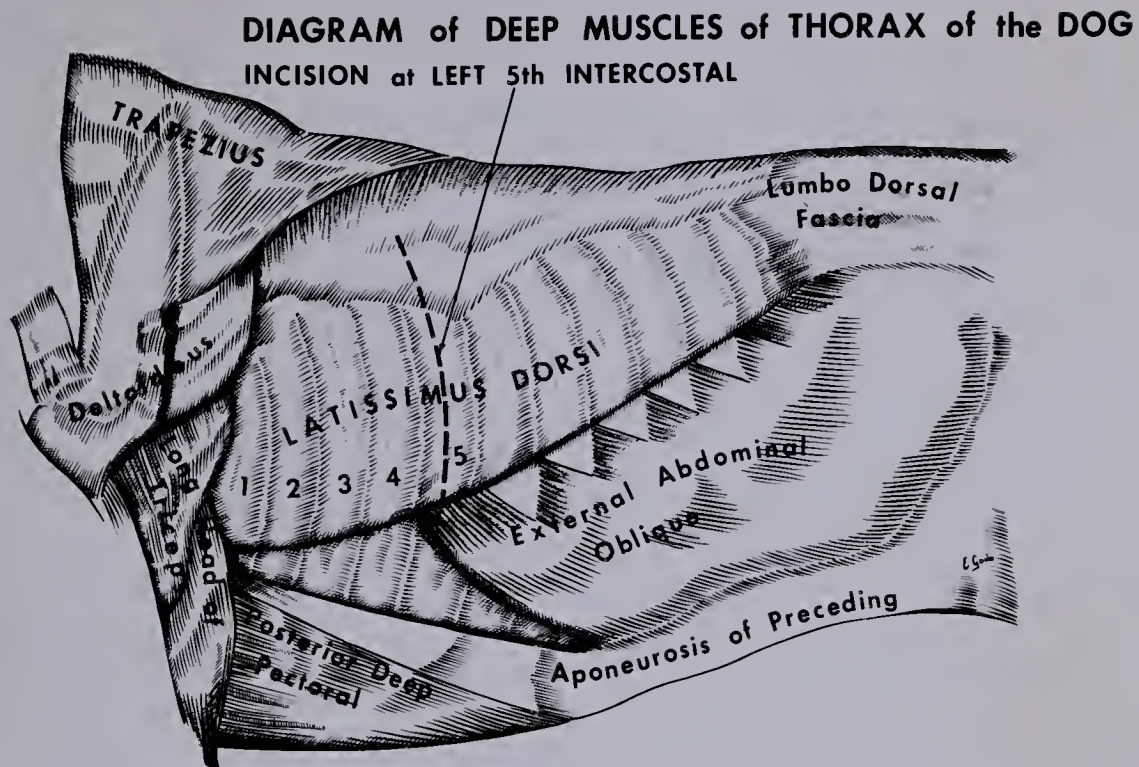
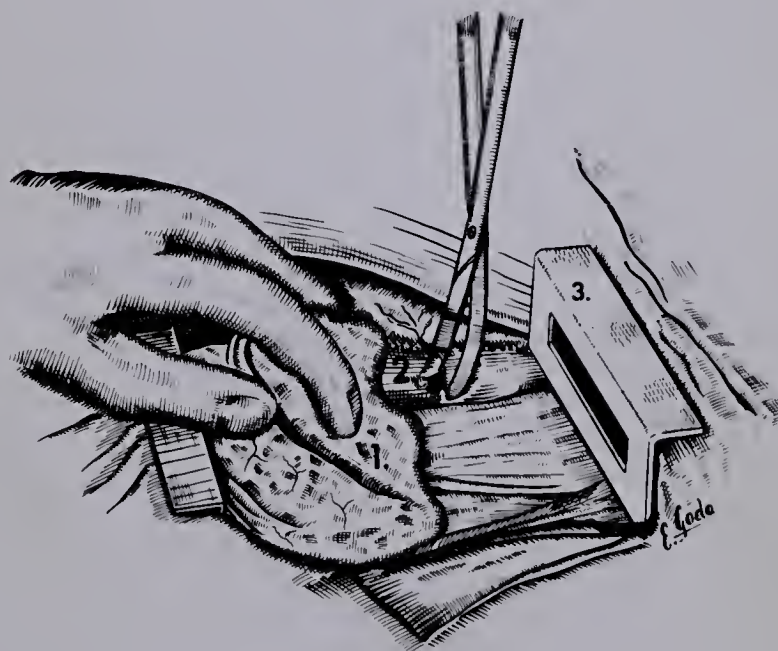


Figure 1. Left Lung Autotransplantation - Incision.

### DIVISION of INFERIOR HILAR LIGAMENT



- 1. Left Lung.
- 2. Left inferior hilar ligament.
- 3. rib retractor.

Figure 2. Left Lung Autotransplantation.



continued superiorly, and the left pulmonary artery, mainstem bronchus and the entrance of the pulmonary veins into the left atrium isolated. All nerves, lymphatics and bronchial vessels were divided. During the period of hilar dissection, the lung was periodically allowed to re-expand. The pulmonary artery and bronchus were then divided between atraumatic vascular clamps (Bulldog or Potts ductus clamps) (Figs.3,4). By blunt and sharp dissection a plane of cleavage was created between the right pulmonary artery and left atrium. A modified Satinsky clamp was applied to the left atrium distal to the openings of the left pulmonary veins, special care being taken to provide a sufficient atrial cuff for re-anastomosis, and at the same time avoiding occlusion of the right pulmonary veins. A cuff of atrium containing the entrance to the left pulmonary veins, was then excised just distal to the clamp and the lung removed (Fig. 5).

The vasculature of the excised lung was then flushed by gravity perfusion with a "balanced" solution containing:

Tis-u-sol	500 cc.
6% dextran in normal saline	500 cc.
heparin sodium	100 mgm.

TRISS buffer - a sufficient amount to adjust to pH 7.35, which had previously been cooled to 4°C. Perfusion was continued until the venous return was clear or approximately 400 cc. of perfusate had passed through the lung.

Reimplantation consisted of re-establishing continuity







### LEFT PULMONARY ARTERY DIVISION BETWEEN CLAMPS

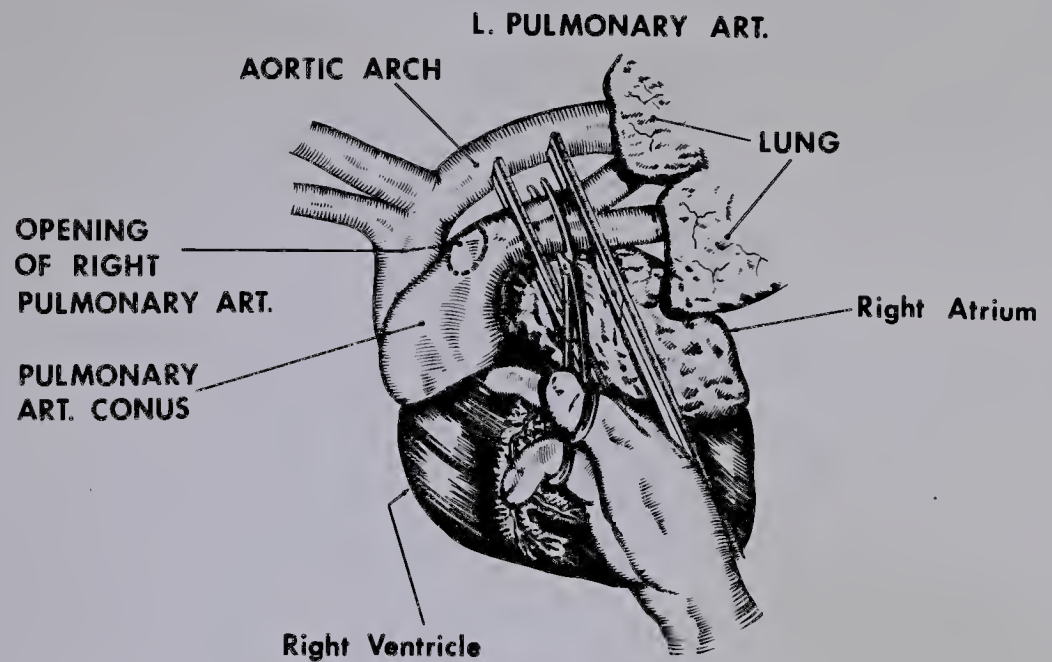


Figure 3. Left Lung Autotransplantation

### DIVISION of LEFT MAINSTEM BRONCHUS

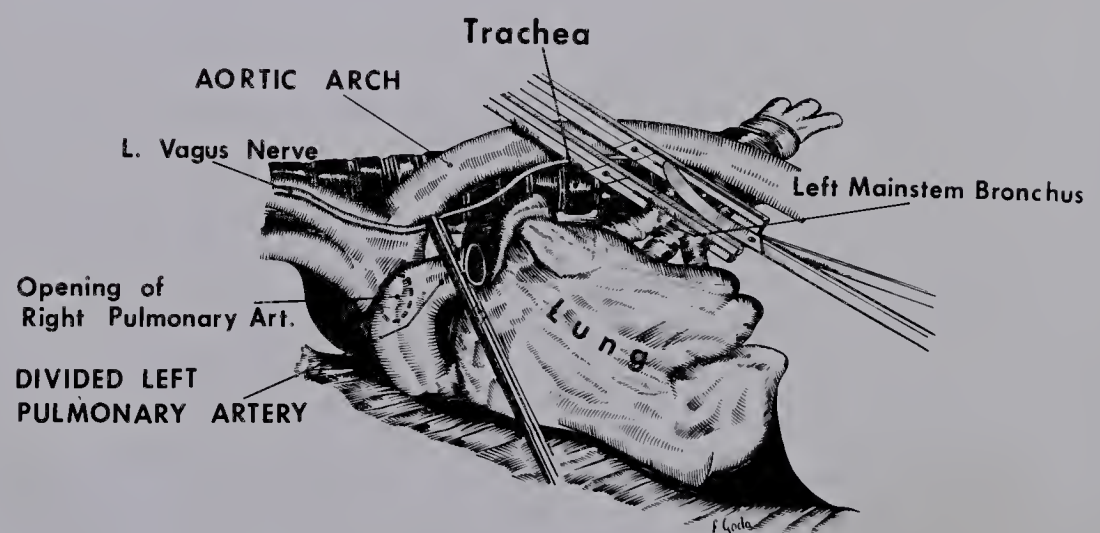
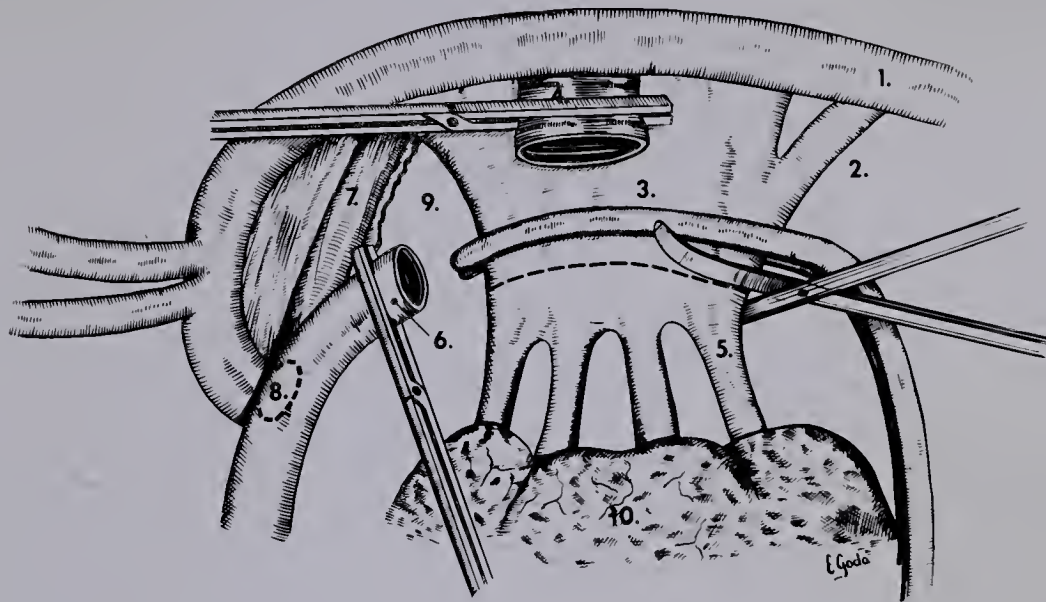


Figure 4. Left Lung Autotransplantation.



## EXCISION of LEFT ATRIAL CUFF



1. Aorta.
2. Inferior Right Pulmonary Vein.
3. Left Atrium.
4. Left Bronchus.
5. Inferior Left Pulmonary Vein.
6. Left Pulmonary Artery.
7. Right Pulmonary Artery.
8. Bifurcation of Main Pulmonary Artery.

Figure 5. Left Lung Autotransplantation.





of the left pulmonary artery, mainstem bronchus, and cuff of atrium. No attempt was made to re-establish continuity of other divided structures. The atrial cuff was reconstituted first with a continuous simple 000000 previously siliconized mersalene or 000000 silk suture (Fig. 6). The anastomosis was begun opposite the inferior pulmonary vein, then continued first inferiorly, and then superiorly, being careful to obtain good endothelial approximation. No stay sutures were placed, since this made exposure of the inferior portion of the anastomosis difficult. The bronchial and arterial anastomoses were carried out in a similar fashion, using 0000 siliconized silk and 000000 mersalene or 000000 silk respectively. Sponge packing was placed about the vascular anastomoses, the clamps were released in the following order: atrial, bronchial and arterial (Figs. 7,8,9). It was found early in the study that if the pulmonary artery clamp was released before the bronchial or atrial clamps, graft edema ensued.

The lung was allowed to inflate undisturbed for approximately five minutes to secure hemostasis. Total graft anoxia time from clamping the pulmonary artery to the release of all clamps, was from 45 to 90 minutes. The packing was then removed and if excessive bronchial air leakage or hemorrhage occurred, additional sutures were placed, care being exercised not to compromise the lumina. Once the technique was reasonably well mastered, additional sutures were not required. The intravenous infusion was



### LEFT ATRIAL ANASTOMOSIS

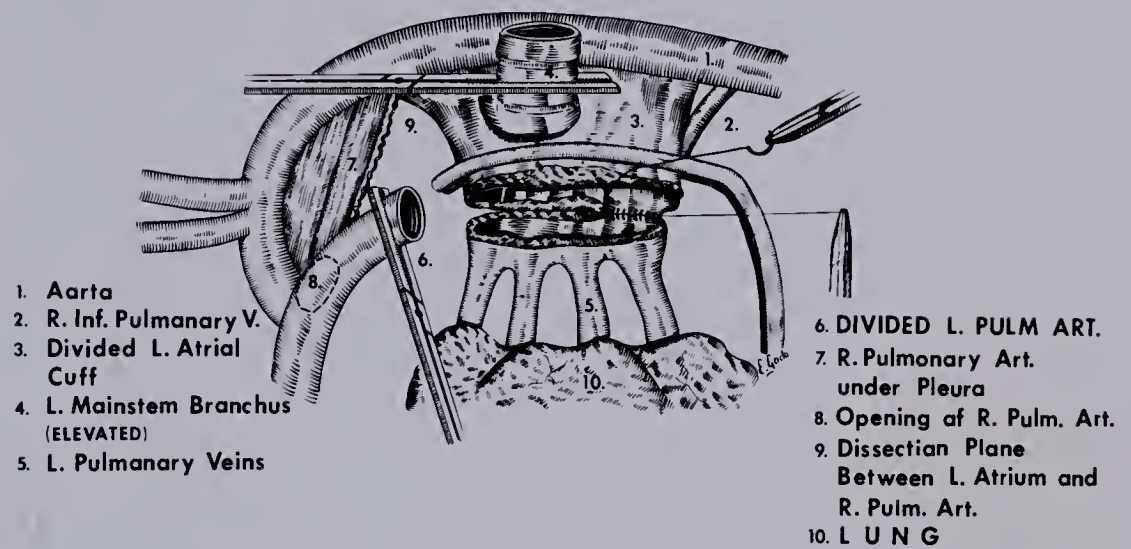
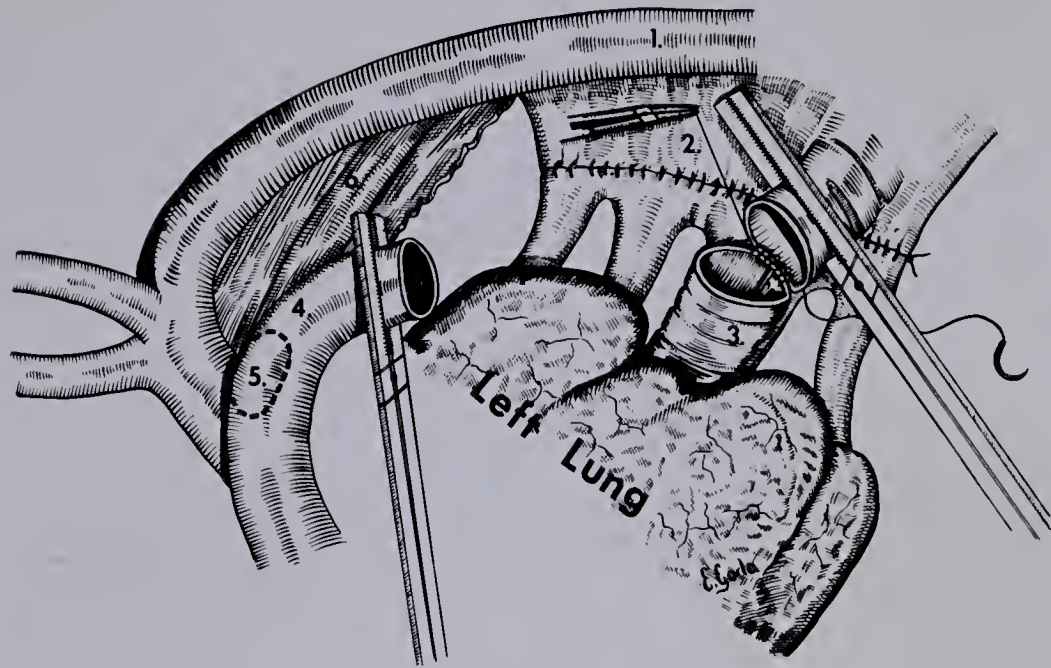


Figure 6. Left Lung Autotransplantation - Atrial Anastomosis.





## BRONCHIAL ANASTOMOSIS

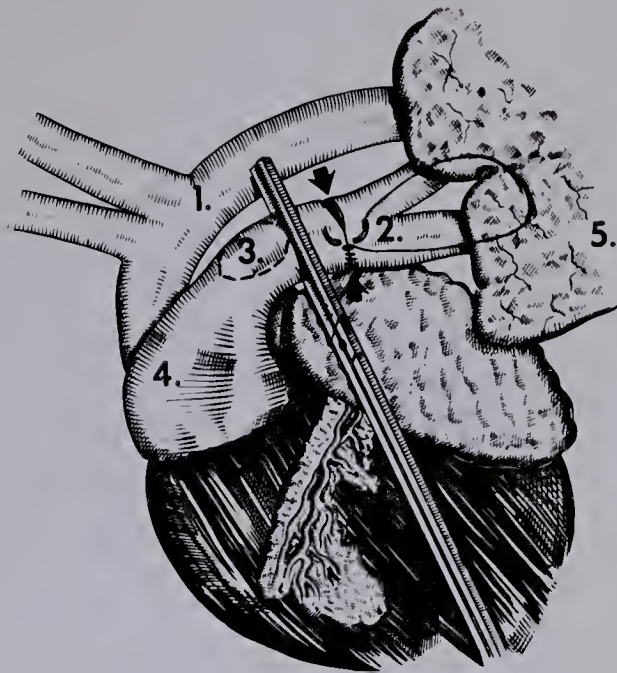


1. Descending Aorta.
2. Left Atrium.
3. Left Mainstem Bronchus.
4. Left Pulmonary Artery.
5. Opening of Right Pulmonary Artery.
6. Right Pulmonary Artery.

Figure 7. Left Lung Autotransplantation -  
Bronchial Anastomosis.



## PULMONARY ARTERY ANASTOMOSIS



1. Aortic Arch.
2. Left Pulmonary Artery.
3. Opening of Right Pulmonary Artery.
4. Pulmonary Artery Conus.
5. Left Lung.

Figure 8. Left Lung Autotransplantation -  
Arterial Anastomosis.





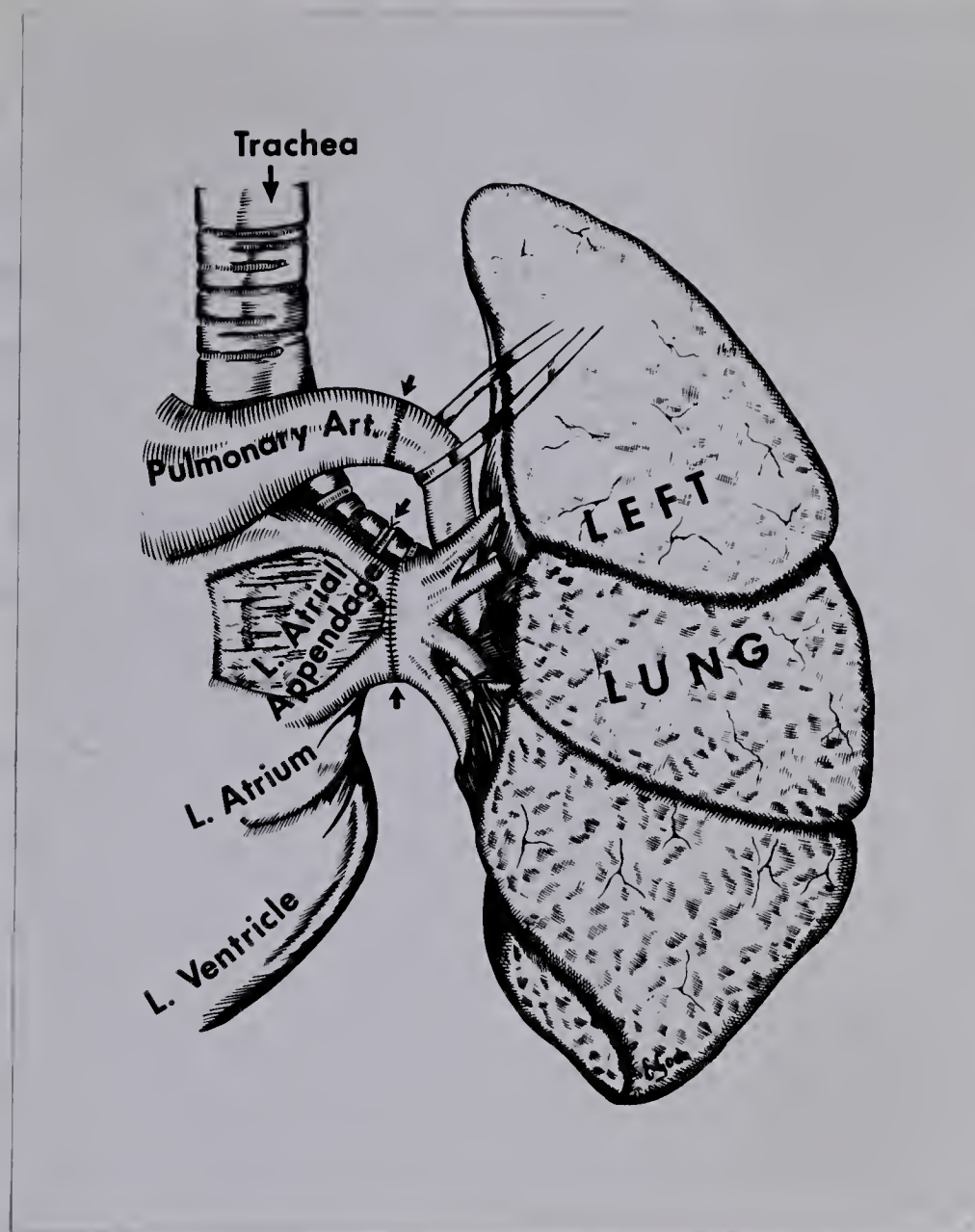


Figure 9. Left Lung Autotransplantation - Relationship of Suture Lines.



switched to 6% dextran in normal saline. A bardec 9 chest tube was inserted through a seventh interspace stab wound and attached to under-water drainage. The chest was then closed in layers in the conventional manner.

Any residual pneumothorax present at the end of the procedure was removed via the chest tube with gentle suction. The respirator was changed to deliver assisted, rather than controlled positive pressure and then when the animal appeared to be respiring spontaneously, was discontinued. The dextran infusion was discontinued after approximately 250 cc. had been delivered. Tracheal aspiration was performed via the endotracheal tube for approximately one-half hour, after which the tube was removed. Additional tracheo-bronchial toilet could be performed subsequently with the aid of a laryngoscope if needed. If excessive bloody fluid was aspirated from the tracheobronchial tree, 10 to 20u of ACTH or 50 mgm. hydrocortisone were given I.V. as prophylaxis against aspiration pneumonia.

b) Post-operative care: All dogs received 200 mgm. tetracycline daily for seven days. On day one, post-operative, chest x-ray was done to evaluate pneumo-hemothorax. If the lung was satisfactorily expanded, the chest tube was removed, due to difficulties in maintaining dogs on chest drainage for prolonged periods. Later, when more experience was gained, early graft status was evaluated by chest examination only. Skin sutures were removed on the seventh post-operative day.





## 2. HYPOTHERMIC GRAFT PRESERVATION.

a) Surgical Technique: The twenty-two mongrel dogs comprising this group were subjected to delayed autologous left lung transplantation. Animal preparation, thoracotomy and graft removal were performed in the manner outlined above, except that the graft was maintained inflated during the period out of the body by clamping the bronchus at the end of inspiration. A sterile probe attached to a direct reading thermistor\* was inserted into the lung parenchyma. The lung vasculature was then flushed in the usual manner. The lung was placed in a sterile bowl containing 5% glucose in saline buffered with TRISS to a pH of 7.35 previously chilled to 4°C and covered with sterile sponges soaked in the same fluid to prevent dessiccation. The bowl was wrapped in a sterile drape and placed in the refrigerator. Lung parenchymal temperature was monitored and recorded at 15, 30 and 60 minutes, and hourly thereafter, and was maintained between 5 and 10°C. When graft temperature was allowed to fall below 5°C, results were discarded.

The pulmonary artery, bronchial and atrial clamps were left in place until reimplantation was completed. For 24 hour procedures, this technique was modified slightly. After pneumonectomy, the proximal left atrial stump was closed with a simple over and over 000000 silk suture. The distal atrial cuff was later anastomosed to the left atrial appendage. The left

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\* Obtainable from Yellow Springs Instrument Co., Incorp., Yellow Springs, Ohio, U.S.A.





pleural cavity was packed with a laparotomy pad soaked in buffered 5% glucose and saline, and the chest closed over water-seal drainage. Metal clips were used to facilitate skin closure. For short preservation procedures, anesthesia was maintained with Atravet and the animals were kept on assisted or controlled respiration.

Total anoxia times and numbers of animals in each group were as follows:

TABLE I.

Dogs subjected to various total graft anoxia times at 5-10°C.

Group	No. of dogs	Total Anoxia Time
I	3	24 hours
II	2	10 hours
III	2	8 hours
IV	7	6 hours
V	8	4 hours

One hour before the predetermined total graft anoxia time, the chest was reopened and the pack removed. If the vascular or bronchial pedicles appeared to be hemorrhagic or necrotic, the clamps were replaced proximally and the necrotic portion resected. The graft was then removed from its container, reimplantation performed and the chest closed in the previously described manner. The period of reimplantation was timed to coincide exactly with the predetermined total anoxia time.





b) Post-operative care: In addition to the previously described post-operative measures, dogs subjected to delayed reimplantation required increased care. Because of the necessity in most cases of prolonged anesthesia and analgesia, Megamide (100 mgm.) I.V. was given at the completion of the procedure to counteract barbiturate respiratory depression. If pulmonary edema developed, constant tracheal suction was necessary for as long as three hours post-operative. Initially, those dogs receiving systemic heparinization were given 1.5 mgm. heparin sodium (1/1,000 solution) I.V. at the completion of the procedure and q4h for 72 hours post-operative. Later, heparinization was started two hours prior to reimplantation with 1.0 to 2.0 mgm./kg. heparin sodium (1/1,000 solution) 1 M and q8h for 72 hours. The majority of animals receiving grafts anoxic for four hours were not heparinized.

All animals dying post-operatively were autopsied within a short time of death. Only the intrathoracic contents were examined. The animal was examined grossly for evidence of pulmonary edema froth, aspiration of vomitus, subcutaneous emphysema and wound healing. The thoracic cavity was widely exposed by removing the anterior chest wall. The degree of pneumothorax and intrathoracic bleeding were assessed. Initially, only the graft with its anastomoses intact, was removed. Later the entire intrathoracic contents were removed and examined. The three anastomoses were opened to assess patency, and portions of them together with portions





of all pulmonary lobes bilaterally were submitted for histologic examination.

### 3. COMBINED HYPERBARIC OXYGENATION AND HYPOTHERMIA PRESERVATION.

a) Apparatus: For this study a hyperbaric chamber specially modified for lung preservation was used.\* The chamber consisted of a small autoclave, to which were added a special organ chamber, a perfusion pump, a respirator and a cooling system. The cooling system consisted of a small conventional refrigeration unit, mounted outside the chamber connected via pressure-tight fittings to approximately 20 feet of 1/2" copper tubing, coiled and mounted within the chamber. A Bird Mark VII respirator, mounted inside the chamber was triggered by an external pressure source. Expired gases were allowed to escape into the chamber and were blown off via a safety valve set at 28 p.s.i. mounted on the top of the chamber. The respirator was fitted with remote controls for sensitivity and inflation pressure to allow external regulation of graft ventilation. Pressurization of the chamber was accomplished through a second, adjustable valve on top of the chamber via a high pressure hose connected to a high pressure compressed air source. Although perfusion was not used in this study, the chamber was fitted with an external ventricle-type pump whose arterial and venous lines passed through pressure fittings in the door of the chamber. The

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\* Built by the University of Alberta Technical Services, and kindly loaned to us for this study by Drs. C. Ross and C. Dafoe, Surgical-Medical Research Institute, University of Alberta.





organ chamber consisted of a stainless steel venous reservoir upon which was mounted a removable perforated stainless steel plate. Upon this plate the lung rested while within the chamber. Strain gauge and thermocouple lines were also passed through pressure fittings in the wall of the chamber. Mounted on the chamber roof was a heat lamp for illumination and graft re-warming.

Special cannulas were manufactured to facilitate bronchial cannulation and rapid coupling to the respirator line.\*

b) Surgical procedure: Prior to the start of the operation, the hyperbaric chamber was cooled to 5°C. Four mongrel dogs weighing 18 to 23 kgs. were anesthetized and prepared in the usual manner. Under sterile conditions, the left lung was removed in the previously described manner, and its vasculature flushed free of blood with "balanced perfusate" cooled to 4°C. The bronchus was then cannulated and the graft was placed in a sterile plastic bag containing 250 cc. buffered balanced perfusate. The lung was placed in the hyperbaric organ chamber, the bronchial cannula connected to the respirator line, and ventilation initiated. After checking the cannulation for air leaks, the hyperbaric chamber was sealed and pressurized to 28 p.s.i. (three atmospheres absolute) with compressed air over a period of 10 minutes. Parenchymal graft temperatures could not be monitored because of trauma inflicted upon the lung by the thermocouple probe during inflation and deflation.

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\* Fabricated by the University of Alberta Technical Services, Mr. R. MacDonald.





Chamber temperature, ventilatory pressure and rate, however, were monitored at intervals throughout the procedure. The chest was closed temporarily over water seal drainage in the usual manner. Four hours after the pulmonary artery was clamped, heparin sodium 1/10,000 solution, 1.5 to 2.0 mgm./kg. S Q was administered.

Five hours after pulmonary artery clamping, the left chest was reopened and the chamber decompressed, over 10 minutes (2.8 lb./minute). The lung was removed from the chamber and its sterile bag, and reimplantation begun. Following its completion, the clamps were released, the lung reinflated and the chest closed in the usual manner. Total graft anoxia time was limited to six hours of which approximately 15 minutes was utilized in excising the graft and preparing it, 4 hours 45 minutes under pressure and hypothermia, and one hour in re-warming and reimplantation of the lung. The entire procedure took approximately 7 hours to complete.

Post-operative care was identical to that given dogs subjected to hypothermic lung preservation alone.

#### 4. PULMONARY FUNCTION STUDIES.

All experimental animals surviving 7 post-operative days or longer were deemed chronic survivors. Assessment of the function of the graft was carried out in the following manner:

a) Pre- and post-operative blood  $\text{PaCO}_2$ ,  $\text{PaO}_2$  and pH determinations: Pre-operative arterial blood samples were





obtained by percutaneous femoral artery puncture either just subsequent to anesthetic induction or after the start of controlled ventilation. Because of wide variation in results, and because normal blood gas values for spontaneously breathing anesthetized dogs were well known from a previous study in this laboratory (252), this procedure was abandoned after completion of the immediate reimplantation group, and only post-operative blood gas determinations were performed.

Post-operative samples were obtained from anesthetized animals in the same manner seven days post-operative, at the time of pulmonary angiography. Samples from anesthetized dogs were obtained simply by percutaneous femoral artery puncture. The procedure was well tolerated. All determinations were done using the Astrup micro-blood gas determination or the Epsco apparatus and method.

b) Chest X-rays: Antero-posterior and left lateral chest x-rays were routinely performed on the first and fourth post-operative days.

c) Pulmonary angiography: All animals were anesthetized with pentothal sodium 30 mgm./kgs. 7 days post-operatively. After surgical preparation of the left side of the neck, they were placed in the right lateral decubitus position on the x-ray table. Under sterile conditions the left external jugular vein was exposed and cannulated with a 50" number 16 French stomach tube,\* previously fitted with a number 13 needle

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\* Obtainable from Becton, Dickinson and Co., Rutherford, N.J. U.S.A.





to facilitate dye injection. The cannula was advanced to the level of the right atrium, or into the heart when possible. Standard antero-posterior (AP) and left lateral chest x-rays were then taken. Twenty ml. of 90% hypaque sodium (sodium diatrizoate)\* was injected through the cannula and approximately three seconds later an AP chest x-ray was taken. The procedure was repeated in the right decubitus position. After removal of the cannula, the vein was either ligated or repaired with 000000 silk. The skin was closed in the usual manner.

d) Alveolar-arterial CO<sub>2</sub> gradients: Dogs were anesthetized in the usual manner using pentothal sodium 30 mgm./kg., 7 days to 3 months post-operatively. The method was established using five normal mongrel dogs 15 to 25 kgm. in weight. An endotracheal tube was inserted and the cuff inflated.  $FI_{CO_2}$  and  $FE_{CO_2}$  were monitored continuously during spontaneous respiration, using a Beckman infra-red instantaneous CO<sub>2</sub> analyzer. After a few minutes were allowed for the animal to achieve a reasonably steady state of gas exchange, an arterial blood sample was withdrawn over a two minute period by percutaneous femoral artery puncture and analyzed for PaCO<sub>2</sub> using an EPSCO blood gas determination apparatus.  $FE_{CO_2}$  was recorded continuously over the period of blood sample removal. Apparatus calibrations were checked between each procedure.

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\*Obtainable from Winthrop Laboratories, Aurora, Ontario.





e) Right heart catheterization: Two weeks to two months post-transplant, all survivors were again anesthetized with pentothal sodium and placed in the dorsal recumbent position. The right neck was prepared and under sterile conditions the right external jugular vein exposed. Intracardiac pressures were measured with a number 6 F. cardiac catheter connected to a Statham strain gauge and Sanborn recorder. After venous cannulation the catheter tip was advanced medially and caudally until right atrial pressure tracings were recorded. The catheter tip was then rotated  $90^{\circ}$  inferiorly and advanced caudally. When right ventricular pressures were recorded, the tip was rotated  $180^{\circ}$  medially and advanced once again. After recording pulmonary artery pressures, right ventricular, right atrial and systemic venous pressures were recorded as the catheter was withdrawn. No fluoroscopy was used. The entire procedure usually took less than 15 minutes, and blood loss was minimal.

f) Alveolar-arterial oxygen gradients: The anesthetized dogs were intubated with a cuffed endotracheal tube and the cuff inflated. The endotracheal tube was attached to a non-rebreathing valve, inspiration being drawn from room air and expiratory gas being collected in a standard 6 liter spirometer, previously flushed three times with the dog's own expiration. Before and after collection of the expired gas,  $\text{FECO}_2$  was monitored using the previously described apparatus. Simultaneously, an arterial blood sample was withdrawn from the





femoral artery over a three minute period. The animal was then disconnected from the non-rebreathing valve and the mixed expired gas was analyzed for  $FE_{CO_2}$  and  $FE_{O_2}$ . The latter measurement was accomplished with either an Epsco gas analysis apparatus, or a Beckman  $O_2$  gas analyzer. The arterial blood sample was analyzed for  $Pa_{O_2}$  and  $Pa_{CO_2}$  using the Epsco apparatus.  $FA_{O_2}$  was calculated using the following formula:

$$F_{A_{O_2}} = F_{I_{O_2}} - \frac{F_{A_{CO_2}}}{F_{E_{CO_2}}} (F_{I_{O_2}} - F_{E_{O_2}}) \quad (221).$$

Initial observations indicated that pentothal produced a large increase in A-a  $O_2$  gradient. The anesthetic was then changed to 3 cc./kgm. body weight of solution containing 3% chloralose and 10% urethane, or 10% chloralose in 25% ethyl alcohol.

g) Differential bronchspirometry: Several methods for separating the ventilation from the two lungs in the dog were tried before a satisfactory one was evolved. The standard Carlens differential bronchspirometry tube as suggested by Hardy (93,94) and the tracheal divider suggested by Trummer (118) for these studies were tried, but gave inconsistent results. If good separation of ventilation was obtained, bronchial obstruction often occurred. If the tube was withdrawn slightly to alleviate the obstruction, leakage of gas from one system into the other across the carina occurred. These disadvantages were not seen as frequently when the White or reverse Carlens differential bronchspirometry





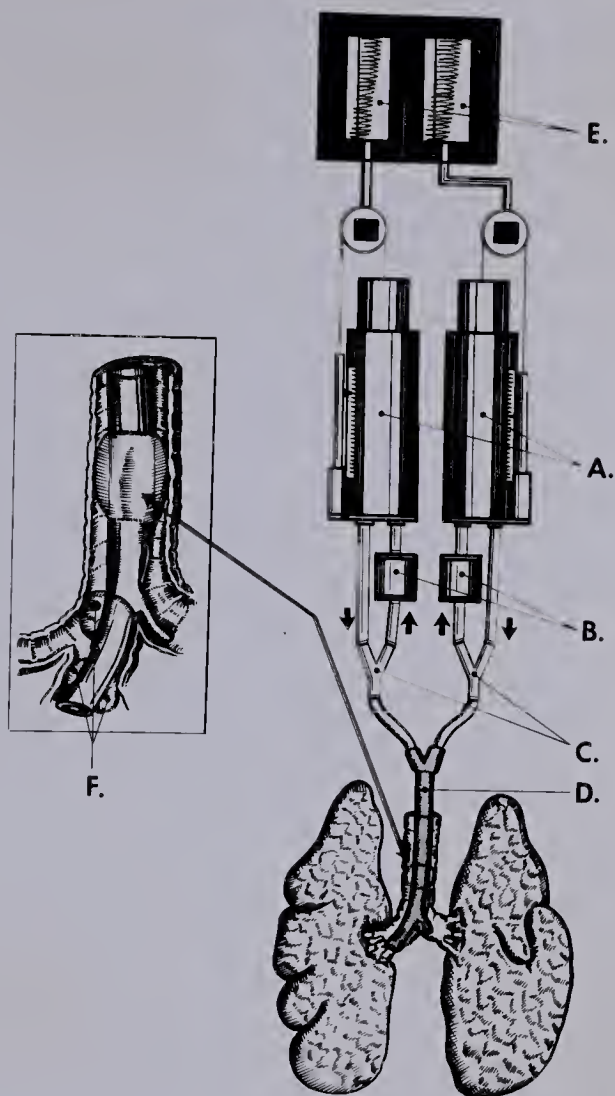
tube was used (Fig. 11). Two water filled six liter spirometers (Fig. 10) were filled with pure oxygen, and connected to a non-rebreathing valve in such a way that inspiration came directly from the spirometer and expiration passed back to the spirometer through a CO<sub>2</sub> absorber. All lines were flushed with oxygen and clamped. Under nembutal anesthesia and aseptic conditions a temporary transverse tracheostomy was performed at the level of the third tracheal ring. A White\* tube was inserted through the tracheostomy opening and advanced until a firm resistance was felt. The cuffs were inflated and the ventilation through each channel assessed grossly by observing chest wall movements. One opening was then obstructed in end inspiration. If the respective side of the chest failed to collapse while the cannula was obstructed, absence of leakage across the carina was assumed. The cannulas were connected to the spirometers and ventilation and oxygen uptake was recorded on a physiograph recorder. The spirometers were refilled and the presence or absence of both the right and left Hering-Breuer reflexes ascertained. The line carrying ventilation from the left lung was clamped in inspiration (i.e., the left lung was maintained in inflation, while the right lung was allowed to collapse). The reflex response was recorded in the ventilation of the right lung. After allowing both lungs to be ventilated for a period, the procedure was repeated for the

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\* Obtainable from Rusch and Co. of Canada Ltd., 64 Gerrard St. E. Toronto 2, Ontario.







- a) Six liter water filled spirometers.
- b) CO<sub>2</sub> absorbers.
- c) Non-rebreathing valves.
- d) White tube.
- e) Physiograph recorder
- f) Right bronchial balloon inflated.

Figure 10. Differential Bronchspirometry Apparatus





Figure 11. Tip of White Tube.  
Note opening for right upper lobe bronchus.





right lung.

The White tube was then removed, the tracheostomy closed by suturing only the peritracheal fascia and the skin was closed in the usual manner.

## I S O L A T E D L U N G P E R F U S I O N E X P E R I M E N T S

### 1. SIMULTANEOUS PERFUSION WITH HOMOLOGOUS PLASMA POSITIVE PRESSURE VENTILATION AND HYPOTHERMIA (Group A.).

Fourteen left lungs were studied. The apparatus (Fig. 12) consisted of a special organ chamber\* (Fig. 13), a Med-science roller-type coronary perfusion pump, a Pemco blood filter, a special glass cooling coil\*\* connected to a Swenko gastric hypothermia machine, and a Bird Mark VII respirator. Perfusion pressures were recorded with a Statham strain gauge and Sanborn recorder. Perfusate temperatures were monitored with a Yellow Sands Instrument thermistor. All tubing was 1/4" O.D. Tygon.

Plasma for perfusion was obtained by spinning down heparinized blood obtained from a donor dog. The plasma was either used fresh, or was frozen and thawed the day before experiments were performed.

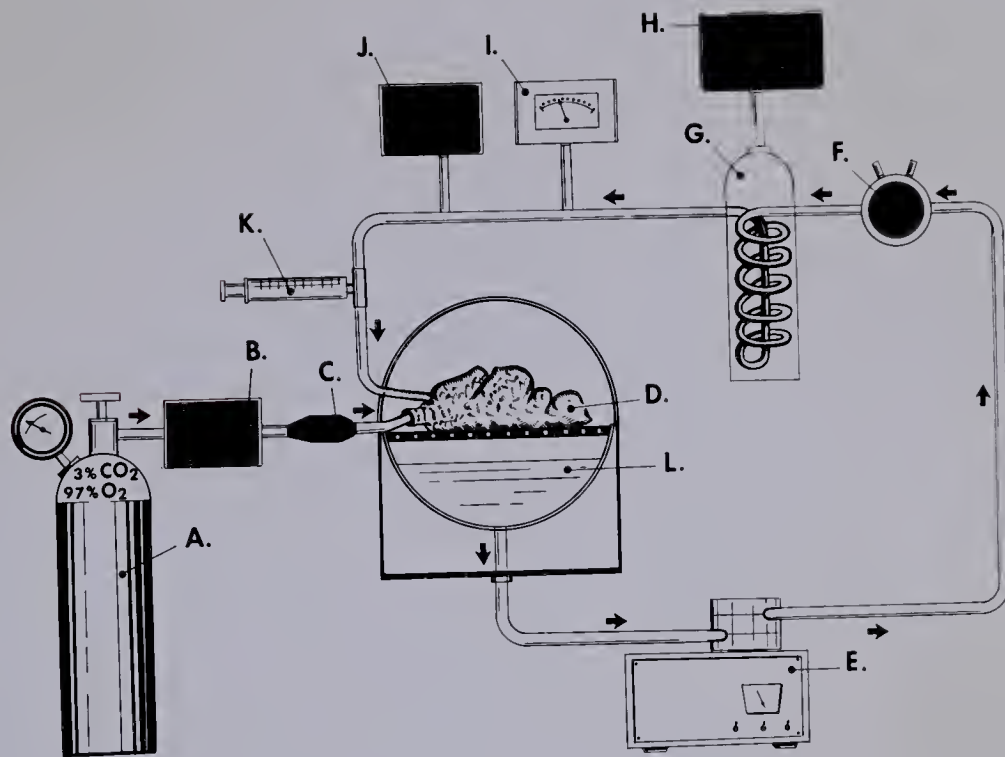
The lungs were excised in the previously described manner, care being taken to leave generous cuffs of pulmonary artery

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\* Designed in our laboratory by Dr. K.G. Dritsas, modified by the author for lung perfusion, and fabricated by the Technical Services, Department of Mechanical Engineering, University of Alberta.

\*\* Designed in our laboratory and fabricated by Mr. P. Alexander Department of Physics, University of Alberta.





- a) Ventilating gas mixture tank.
- b) Bird Mark VII respirator.
- c) Humidifier.
- d) Isolated lung.
- e) Coronary perfusion pump.
- f) Filter.
- g) Hypothermia coil.
- h) Gastric hypothermia machine.
- i) Thermistor.
- j) Strain gauge and recorder.
- k) Sampling syringe.
- l) Venous reservoir.

Figure 12. Isolated Lung Experiment A. Apparatus





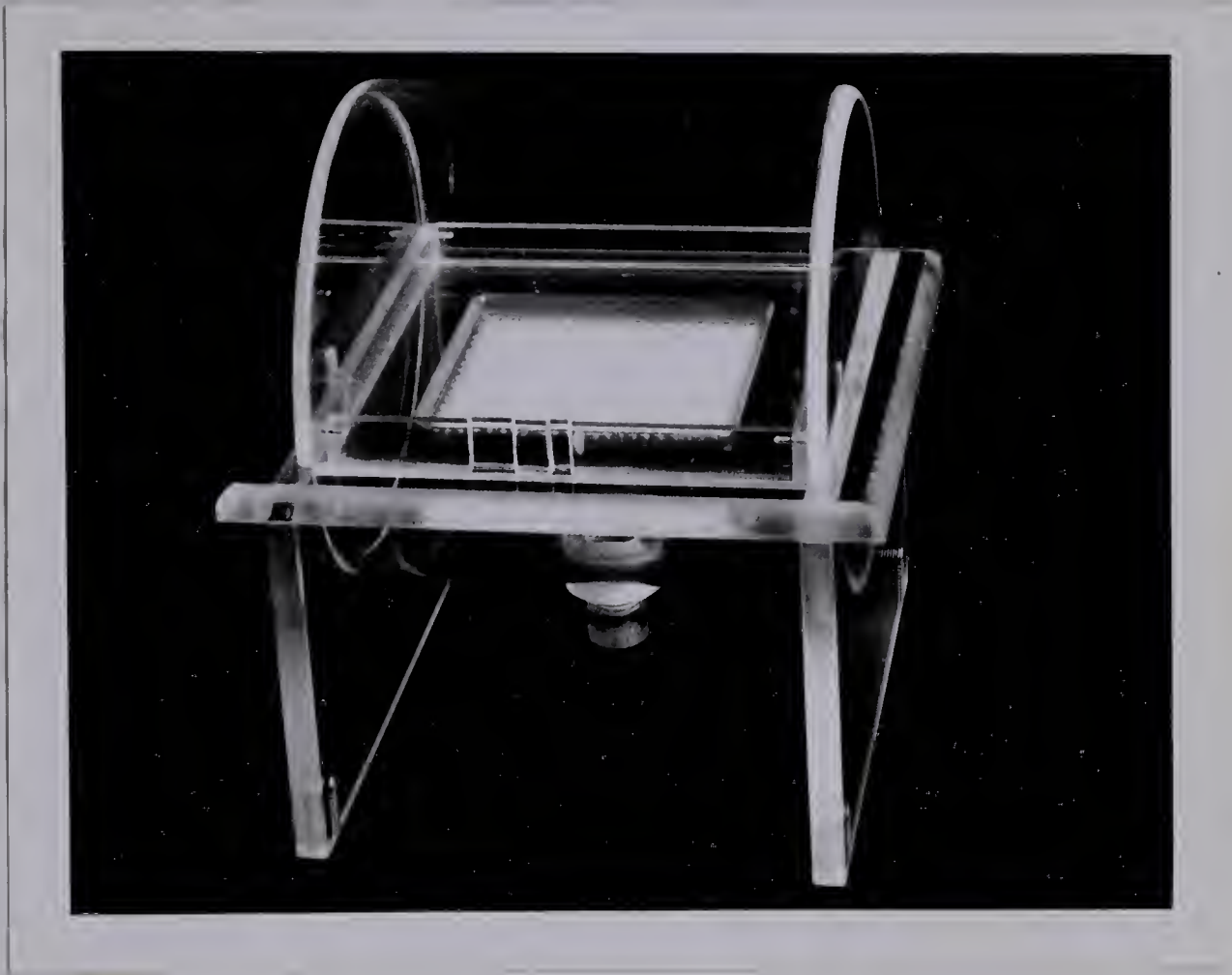


Figure 13. Organ Chamber used in isolated lung Experiment A. Openings in lid allow entry of bronchial and arterial cannulae. Venous return drains by gravity into reservoir.



and bronchus attached to the lung, and weighed. The bronchus was cannulated first and ventilation started with a humidified or non-humidified mixture of compressed air, 97% oxygen or 97% oxygen and 3% CO<sub>2</sub> at 15 cm. H<sub>2</sub>O inflation pressure and 20 cycles per minute. The main left pulmonary artery was then cannulated and perfusion instituted. Temperature was maintained at 15°C. Flow was arbitrarily adjusted to produce an approximation of normal pulmonary artery pressure. At intervals of 0, 15, 30, 60, 120 and 180 minutes, flow, perfusion pressure, temperature and ventilation pressure were recorded. At the same time plasma samples were withdrawn for P<sub>O</sub><sub>2</sub>, PCO<sub>2</sub>, pH, Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, histamine, glucose and total protein determinations. Perfusion time was limited to three hours. The lung was then reweighed and tissue samples taken for Na<sup>+</sup>, K<sup>+</sup>, Cl<sup>-</sup>, histamine, hexosamine and hydroxyproline as shown in Figure 16.

## 2. SIMULTANEOUS PERFUSION WITH AUTOLOGOUS BLOOD, NEGATIVE PRESSURE VENTILATION AND HYPOTHERMIA (Group B.).

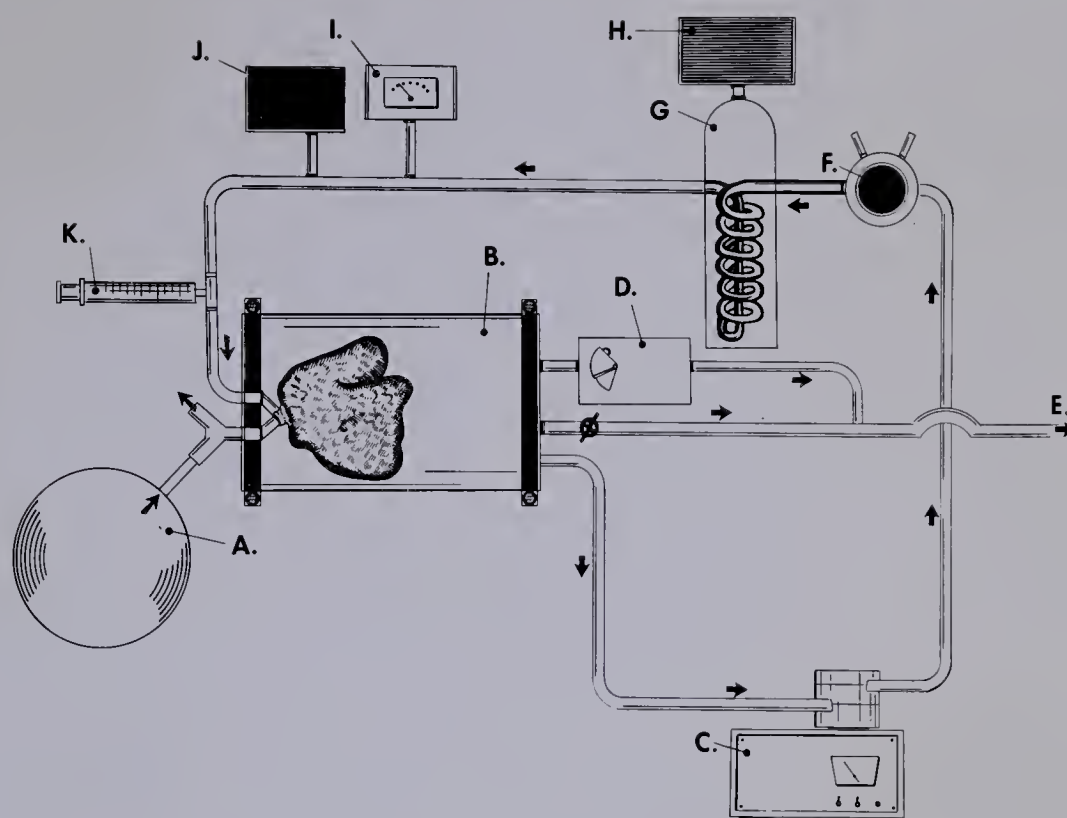
Twelve left lungs were studied under essentially the conditions outlined above, except that two conditions were varied. Negative pressure ventilation was achieved with a special organ chamber\* (Figs. 14,15). One outlet from the chamber was connected directly to a wall vacuum source and maintained -1 to -2 cm. H<sub>2</sub>O pressure in the chamber in expiration. A standard respirator was again connected

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\* Designed in our laboratory and fabricated by Mr. H. Golls, Technical Services, Department of Mechanical Engineering, University of Alberta.







- a) Douglas balloon.
- b) Organ chamber.
- c) Coronary perfusion pump.
- d) Respirator.
- e) Wall vacuum source.
- f) Filter.
- g) Hypothermia coil.
- h) Gastric hypothermia machine.
- i) Thermistor.
- j) Strain gauge.
- k) Sampling syringe.

Figure 14. Isolated Lung Experiment B. Apparatus



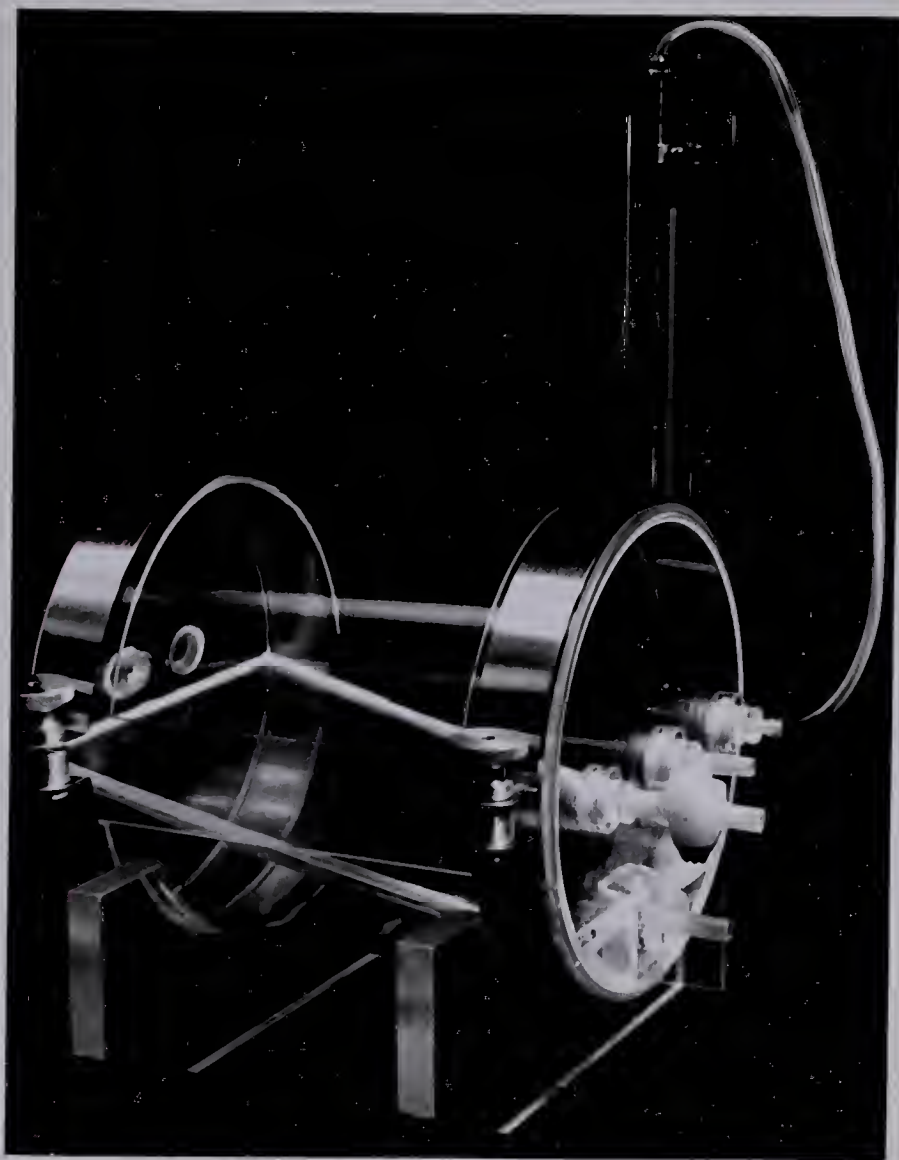


Figure 15. Isolated Lung Experiment B. - Organ perfusion chamber





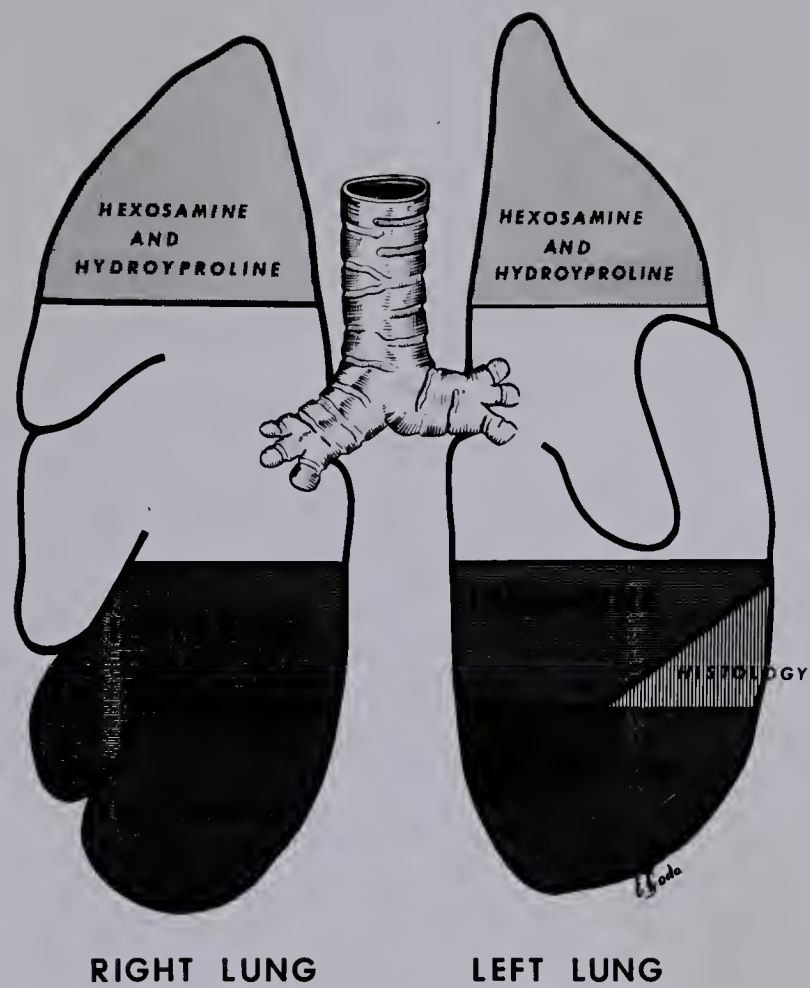


Figure 16. Isolated Lung Experiments A and B.  
Tissue Biochemistry Sampling Sites



directly to the vacuum and cycled the chamber pressure to -10 to -14 cm. H<sub>2</sub>O at a rate of 20 cycles per minute. The humidified 97% O<sub>2</sub> and 3% CO<sub>2</sub> gas mixture was inspired from a Douglas bag via a non-rebreathing valve and bronchial cannula, and expired into a six liter spirometer. Based on experience gained in the previous isolated lung experiments, several modifications in design were incorporated into the negative pressure chamber. Chamber cannula openings were made so that the pulmonary artery and bronchial cannulas would enter the lung at a 90° angle to each other. Also, the floor of the chamber was made smooth, to avoid lung trauma during inflation and deflation. Cannulas were constructed so as to allow cannulation outside the chamber. They could rapidly be coupled to the lines entering the chamber, allowing a reduction in lung anoxia time.

Autologous blood (approximately 1000 cc.) for perfusion was obtained from the dog at the time of lung removal. The pump was primed and the blood cooled prior to placing the lung in the perfusion chamber. Time elapsing from clamping the pulmonary artery to reinstitution of ventilation and perfusion was approximately five minutes. Parameters studied were the same as those studied in isolated lung Experiment A.





## CHAPTER III

### RESULTS AND DISCUSSION



## 1. OPERATIVE MORTALITY.

a) Immediate Reimplantation Group: Approximately 25 dogs were sacrificed or died early in the study in the process of learning the lung reimplantation technique. The principal causes of death included:

- i. Operative hemorrhage (usually from the atrial anastomosis)
- ii. Pneumothorax;
- iii. Stenosis of the pulmonary artery anastomosis with subsequent vascular thrombosis;
- iv. Aspiration pneumonia, and
- v. Acid pH of the vascular flushing solution.

With respect to the last factor, after all technical operative difficulties had seemingly been overcome, chronic survivors were still not obtained. It was then discovered that the pH of the "perfusion solution" was 6.0. After this was adjusted to 7.35, the next eight animals subjected to immediate reimplantation survived chronically. Of these animals, two died late post-operatively, both due to sequelae of left heart catheterization.

b) Hypothermic Preservation Group: The lung parenchymal temperatures measured in five experiments are shown in Figure 17. Perfusion with a solution of 4% effected rapid cooling to 20°C. Further cooling occurred over the next several hours by convection and radiation. Oxygen consumption falls logarithmically with temperature (156,159,160). Therefore, in theory, lung oxygen requirements were reduced to approx-





imately 25% of normothermic requirements within five to ten minutes. Further cooling to 10°C reduced oxygen consumption to about 5% of normal (Fig. 17). The fates and causes of death of dogs subjected to left lung hypothermic preservation and subsequent reimplantation are summarized in Table 2.

All dogs whose lungs were subjected to hypothermic anoxia times of greater than six hours died. One animal in the six hour group survived for a mortality rate of 85.6%. Survival in the four hour group was 100%.

The cause of death in those animals succumbing to the procedure is difficult to ascertain, but some of the following factors appeared to play a role.

i) loss of alveolar surfactant - alveolar surfactant is a non-cellular substance, probably lecithin, normally present within alveoli which lowers alveolar surface tension during inflation, and is responsible for many of the normal static mechanical properties of the lung (222,223). This substance is lost in pulmonary edema, vagotomy and interruption of pulmonary circulation in an increased pressure requirement for normal lung inflation. Pulmonary hemodynamics are also altered (222,223). Following prolonged preservation procedures, froth was observed to pour out of the bronchial stump of the cooled lung even before the vascular clamps were released.\* Post-operative positive pressures of up to 40 cm. H<sub>2</sub>O were required

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\* Also observed by Dr. C. Ross in left lungs subjected to hypothermia at 5°C and HPO of 3 atmos. for 2 hours. (Personal communication).



# LUNG PARENCHYMAL TEMPERATURE - HYPOTHERMIA INDUCED by PERFUSION and SURFACE COOLING

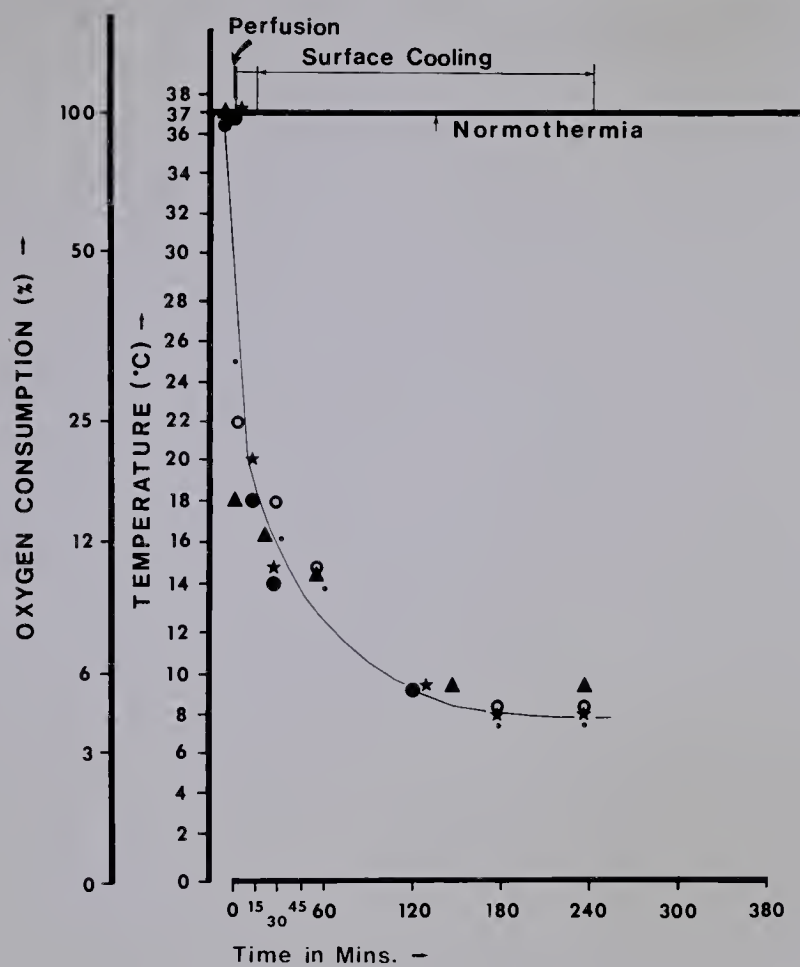


Figure 17. Hypothermic Lung Preservation





TABLE 2

TREATMENT AND MORTALITY

Left Lung Hypothermic Preservation and Reimplantation

Group and Total Lung Anoxia Time	Dog No.	Heparinization	Result
I (24 hours)	512	None	Died 6th P.O. day. Thrombosis artery and veins, hemorrhagic necrosis of lung (T.A. and V., H.N.)
	514	1 mgm/kg q4h 1.m	Died 7th P.O. day. (T.A. and V., H.N.)
	519	1.5 mgm/kg q4h 1.m	Died 5th P.O. day. (T.A. and V., H.N.)
II (10 hours)	663	1.5 mgm/kg q4h 1.m	Died 2nd P.O. day. Bronchial slough, mass- ive pneumothorax.
	644	1.5 mgm/kg q4h 1.m	Died 1 hour P.O. Artery patent, L. atrial thrombosis; pulmonary edema.
III (8 hours)	574	1.5 mgm/kg q4h 1.m	Died 5th P.O. day. (T.A. and V., H.N.)
	602	1.5 mgm/kg q4h 1.m	Died 4th P.O. day. (T.A. and V., H.N.)
IV (6 hours)	524	1.0 mgm/kg q4h 1.m	Died 1st P.O. day. (T.A. and V., H.N.)
	591	1.0 mgm/kg q4h 1.m	Died 5th P.O. day. (T.A. and V., H.N.)
	634	2.0 mgm/kg q4h 1.m	Died 7th P.O. day. (T.A. and V., H.N.)

.....continued.....



TABLE 2. (Cont.)

Group and Total Lung Anoxia Time	Dog. No.	Heparinization	Result
IV (cont.) (6 hours)	814	1.0 mgm/kg 2 hours prior to operation then 1.0 mgm/kg subcut. q8h.	Died 3rd P.O. day. (T.A. and V., H.N.)
	808	1.0 mgm/kg 2 hrs. prior to op. then 1.0 mgm/kg subcut. q8h.	Died 5th P.O. day. (T.A. and V., H.N.) Aspiration pneumonia right lower lobe.
	786	1.0 mgm/kg 2 hrs. prior to op. then 1.0 mgm/kg subcut. q8h.	Died 7th P.O. day. (T.A. and V., H.N.) Aspiration pneumonia right lung.
	816	1.5 mgm/kg & 2.0 mgm/kg q8h subcut.	Died 3rd P.O. day. (T.A. and V., H.N.) Massive bilateral hemothorax.
	795	1.5 mgm/kg & 2.0 mgm/kg q8h subcut.	Died 4th P.O. day. No thrombosis, massive hemothorax.
	815	2.0 mgm/kg & 2.0 mgm/kg q8h subcut.	Died 7th P.O. day. No thrombosis, no hemothorax, H.N.
	612	1.5 mgm/kg q4h.	Survivor
V (4 hours)	596	1.5 mgm/kg & 1.5 mgm/kg q8h subcut.	Survivor
	798	1.5 mgm/kg & 1.5 mgm/kg q8h subcut.	Survivor
	797	None	Survivor

....continued.....





TABLE 2 (Cont.).

Group and Total Lung Anoxia Time	Dog No.	Heparinization	Result
V (cont.) (4 hours)	968	None	Survivor
	873	None	Survivor
	853	None	Survivor
	861	None	Survivor

to maintain the lung inflated. It is therefore postulated that prolonged pulmonary hypothermia in some way damages alveolar surfactant.

ii)thrombosis - although almost every animal which died in the study exhibited both pulmonary arterial and venous or left atrial thrombosis (Fig. 39), with hemorrhagic necrosis of the lung, the etiology of these changes is unclear. The problem resolves itself essentially into one of the suture line versus microvessel thrombosis. That the cause was technical is unlikely, since even large doses of heparin failed to prevent thrombosis and all animals in the four hour group survived, many without any heparinization. Other causes might include:

a) graft vascular stasis due to vasoconstriction or decreased cardiac output as a consequence of prolonged anesthesia.

b) hemoconcentration due to prolonged positive pressure inflation.



c) graft vascular death with alteration of endothelial change.

d) local release of thromboplastin from damaged tissues.

It is possible also that the unoperated right lung suffered from the prolonged clamping of the left atrium with consequent elevation of venous pressure, although there was little evidence to suggest that these changes did in fact occur.

iii) graft death prior to reimplantation - histologic examination of autopsy material revealed complete hemorrhagic necrosis of lungs cooled for 24 hours, 7 days following reimplantation. Alveolar architecture was completely destroyed (Figs. 18,19). Lungs cooled for six hours showed considerable hemorrhage into the organ parenchyma with vascular endothelial alterations, hemorrhage into the muscularis of both the pulmonary artery and atrial cuffs and bronchial epithelial necrosis (Figs. 20,21,22).

iv) post-operative anemia - in the course of assessing post-operative blood loss and hydration, it was discovered that dogs destined to die of vascular thrombosis and graft hemorrhagic necrosis, invariably had a severe anemia (Hb 2.0 to 4.5 gm%), despite the absence of blood loss. On the basis of this finding, coupled with an opaque left lung field and enlarged right heart border on x-ray (Figs. 23,24,25), graft death could be diagnosed with certainty. The etiology of this anemia is unclear, although blood sequestration in the graft and hemolysis are possible explanations. One animal





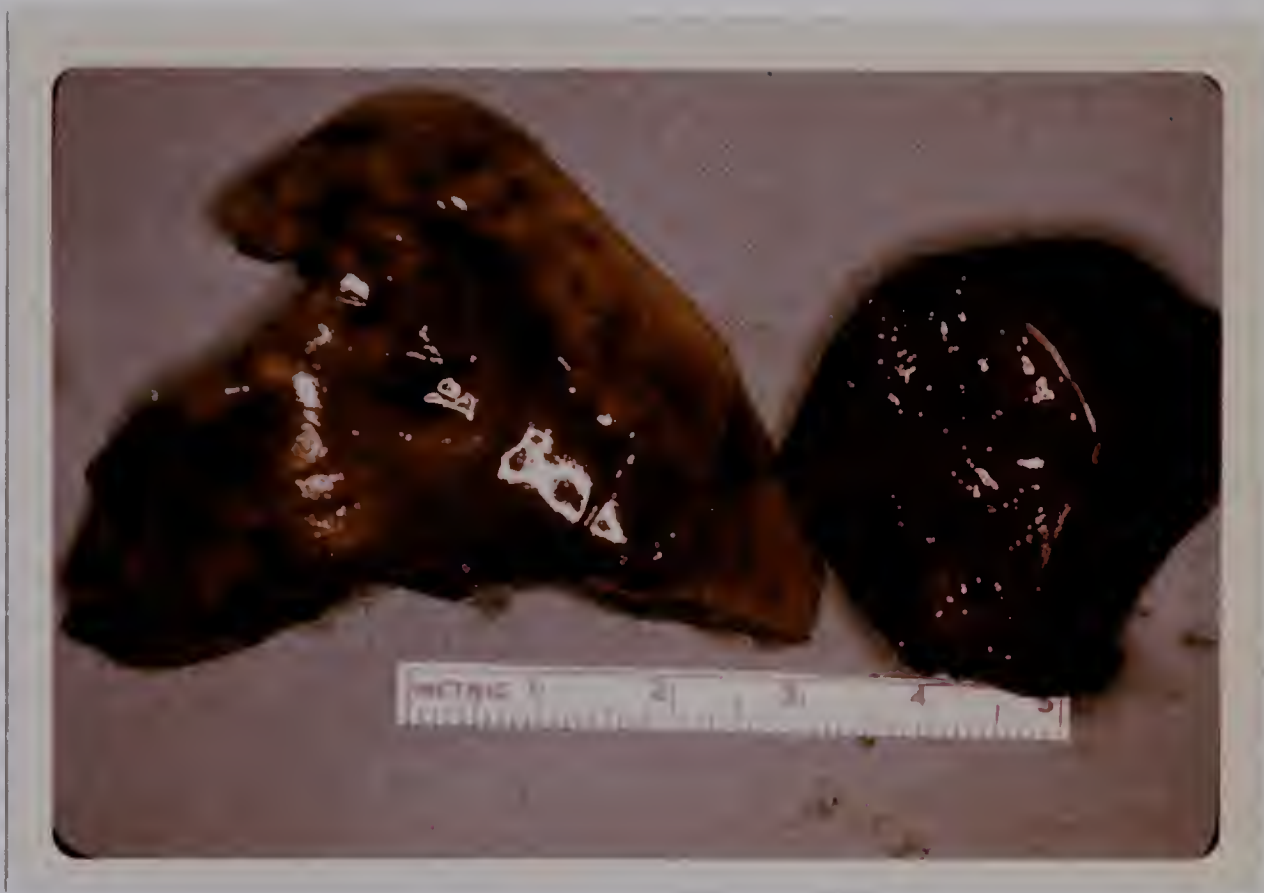


Figure 18. Twenty-four hour Left Lung Hypothermic Preservation. Autopsy specimens seven days following reimplantation; right lung on left, left lung on right.





Figure 19. Twenty-four hour Lung Hypothermia - Lung Histology. Photomicrograph (X 50). Left lung treated with 5° hypothermia for 24 hours and reimplanted. Note the complete loss of bronchiolar and alveolar architecture.





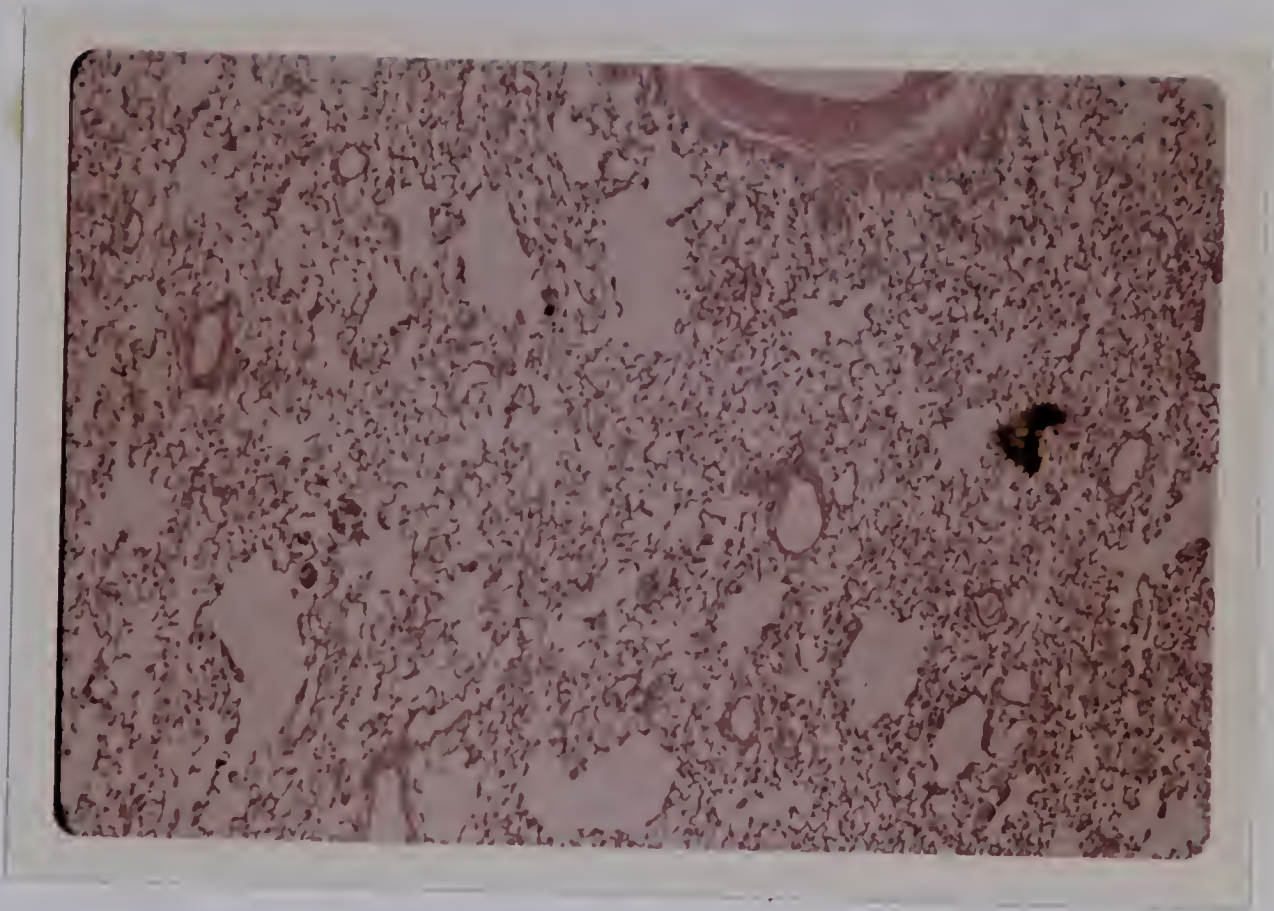


Figure 20. Control Lung - Histology.  
Photomicrograph (X 50). There is  
good aeration and preservation of  
alveolar architecture throughout.





Figure 21. Six hour Lung Hypothermia - Lung Histology. Photomicrograph (X 50) of left lung treated with 5° hypothermia for six hours and reimplanted. Note the considerable intra-alveolar hemorrhage, atelectasis and edema. Some alveolar architecture has been preserved.





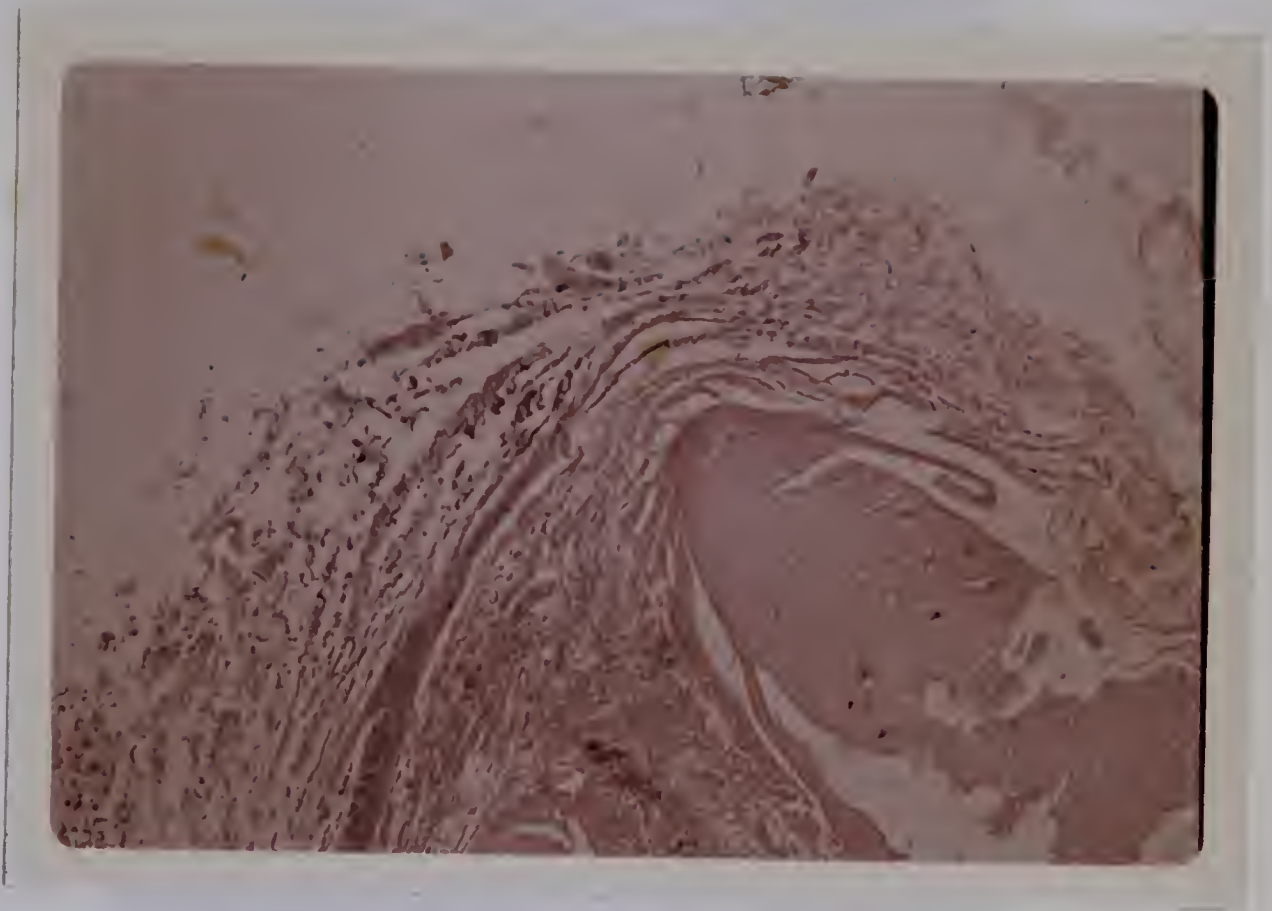


Figure 22. Six hour Lung Hypothermia - Bronchial Histology. Photomicrograph (X 125). Mainstem bronchus from a left lung treated with 5° hypothermia and reimplanted. The bronchial epithelium is necrotic and considerable sub-epithelial infiltration has occurred.



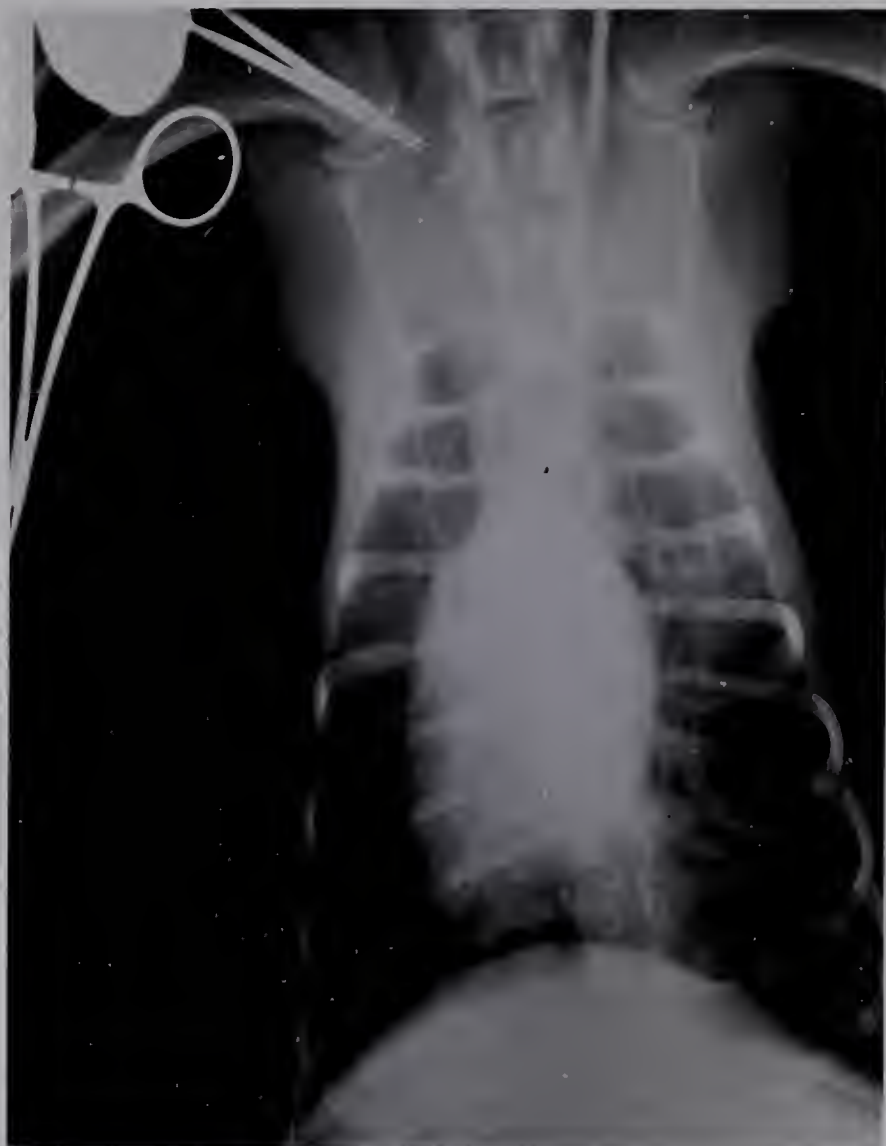


Figure 23. Control Dog AP Chest X-ray.







Figure 24. Ten Hour Hypothermic Lung Preservation. AP Chest x-ray four days following re-implantation. Note increased size right heart border.





Figure 25. AP Chest X-ray - four days following six hour left lung hypothermia preservation. Note increased size right heart border.





subjected to four hour hypothermic preservation died of bilateral pneumonia 3 months post-operatively. Autopsy revealed, in addition to the pneumonia, a lack of significant pleural adhesions (Fig. 35), a well healed, widely patent bronchial, atrial and pulmonary artery anastomoses (Figs 36, 37, 38).

c) HPO and Hypothermia group: Of the four dogs comprising this group, two died within one week post-operatively due to vascular thrombosis and graft hemorrhagic necrosis essentially similar to that described above. One animal died three weeks post-operatively. Autopsy revealed thrombosis of the left atrial cuff with recannulation (Fig. 40), complete left bronchostenosis with atelectasis and bronchiectasis of the entire left lung (Fig. 41). Since this was the only instance of bronchostenosis seen in the series, the cause was not felt to be technical, but rather inflation of the lung inside the hyperbaric chamber with unhumidified oxygen. One animal survived chronically.

Probably most of the complicating factors outlined above also play a role in HPO-hypothermic preservation. However, certain additional problems come into play. It has recently been pointed out (224) that many enzymes, particularly those involved in oxidation of pyruvate, operate best within a narrow range of  $P_{O_2}$ . Most metabolic pathways are inhibited both by lack and excess of oxygen. Applied to organ preservation, this action of HPO could either be a help or hindrance.



Proliferation of capillary tufts, fibrosis and thickening of the alveolar basement membrane may appear within two or three days of HPO treatment in humans (225).

## 2. PULMONARY ANGIOGRAPHY.

All animals surviving chronically save one, showed bilaterally symmetrical pulmonary angiograms (Figs. 26,27,28), although on occasion the outline of the left lung vasculature was not as clear as seen in control angiograms (Fig. 27). These studies demonstrated only grossly adequate graft perfusion and no conclusions as to graft function could be drawn without more definite pulmonary function studies.

## 3. ARTERIAL BLOOD GAS STUDIES.

Control  $P_{aH}$ ,  $P_{aCO_2}$  and  $P_{aO_2}$  are summarized in Table 3. The mean values were as follows:  $P_{aH}$  - 7.39;  $P_{aCO_2}$  - 27.77;  $P_{aO_2}$  - 79.81; and HbO<sub>2</sub> saturation 94.1%.

The values obtained for the same parameters in operated dogs, one week to one month post-operatively, with pentothal anesthesia but breathing spontaneously, are summarized in Table 4. When compared with control values,  $P_{aH}$  and  $P_{aCO_2}$  are essentially unchanged, but the  $P_{aO_2}$  and HbO<sub>2</sub> saturation are considerably lower in the post-operative animals. When samples were taken from unanesthetized post-operative animals, the  $P_{aO_2}$  and HbO<sub>2</sub> saturations compare favorably with control values (Table 5).

The difference in  $P_{aO_2}$  levels of post-operative dogs







Figure 26. Control Pulmonary Angiogram.





Figure 27. Bilateral Pulmonary Angiogram - seven days following immediate reimplantation.





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Figure 28. Unilateral Left Pulmonary Angiogram.  
Dog No. 798, seven days following  
four hour hypothermic left lung pre-  
servation and reimplantation.



TABLE 3

CONTROL ARTERIAL BLOOD GASES

Dog No.	$P_{aH}$	$P_{aCO_2}$	$P_{aO_2}$	$HbO_2$
		(mm. Hg)	(mm. Hg)	sat. (%)
1	7.53	17.0	90.0	97
2	7.39	27.1	100.0	96
3	7.39	25.2	72.0	93
4	7.40	20.5	85.0	96
5	7.34	31.0	64.0	90
6	7.37	35.0	84.0	95
7	7.31	37.0	65.0	90
8	7.37	29.1	84.0	95
9	7.40	21.6	74.0	94
10	7.37	32.0	84.0	95
11	<u>7.40</u>	<u>30.0</u>	<u>76.0</u>	<u>94</u>
x	7.39	27.77	79.81	94.1





TABLE 4

POST-OPERATIVE BLOOD GASES (NEMBUTAL ANESTHESIA)

Group	Dog No.	P <sub>aH</sub>	P <sub>aCO<sub>2</sub></sub> (mm. Hg)	P <sub>aO<sub>2</sub></sub> (mm. Hg)	HbO <sub>2</sub> sat. (%)
<u>Immediate Reimplantation</u>					
	434	7.45	37.9	62.0	93.0
	449	7.34	38.2	58.0	87.0
	356	7.43	41.0	71.0	94.0
	373	7.47	21.5	61.0	91.5
	442	7.32	38.4	47.0	78.0
	<u>411</u>	<u>7.41</u>	<u>27.5</u>	<u>63.0</u>	<u>91.0</u>
	x	7.38	34.0	60.3	91.0
<u>Delayed Reimplantation</u>					
4 hour hypothermia	596	7.38	33.6	40.0	79
	798	7.32	38.8	56.0	84
	797	7.50	28.0	66.0	94
	968	7.36	41.7	50.0	83
	873	7.34	36.5	-	-
	853	7.44	35.2	70.0	93
	<u>861</u>	<u>7.35</u>	<u>33.9</u>	<u>67.0</u>	<u>91</u>
	x	7.38	35.4	58.1	88
6 hour hypothermia	612	7.35	42.0	52.0	83
6 hour hypothermia + OHP	1122	7.34	42.0	56.0	86



TABLE 5

POST-OPERATIVE BLOOD GASES (UNANESTHETIZED)

Group	Dog No.	P <sub>aH</sub>	P <sub>aCO<sub>2</sub></sub>	P <sub>aO<sub>2</sub></sub>	HbO <sub>2</sub> sat. (%)
<u>Immediate Reimplantation</u>					
	434	7.42	27.7	67	94
	440	7.36	32.2	77	94
	356	7.43	31.2	84	96.5
	373	7.35	31.3	81	94.5
	499	7.35	34.8	60.5	88
	<u>442</u>	<u>7.41</u>	<u>28.5</u>	<u>68</u>	<u>93</u>
	x	7.39	30.95	72.92	93.34
<u>Delayed Reimplantation</u>					
4 hour hypothermia	596	7.38	27.1	81	95
	798	7.40	27.9	68	92
	797	7.34	33.6	90	96
	968	7.42	32.7	71	94
	873	7.40	33.6	79	95
	853	7.37	30.9	82	94.5
	<u>861</u>	<u>7.41</u>	<u>27.7</u>	<u>80</u>	<u>96.0</u>
	x	7.39	30.50	78.71	94.64
6 hour hypothermia	612	7.33	38.9	62.0	88
6 hour hypothermia + OHP	1122	7.39	35.5	85	95.5





anesthetized with pentothal and unanesthetized is probably due to alteration in  $\dot{V}_A/\dot{Q}$  produced by the pentothal.

Barbiturates are known to depress the central vasomotor center, but probably do not affect cardiac output per se, but hypoxia secondary to respiratory depression has been incriminated in the etiology of the untoward cardiovascular effects of these drugs (226). Decreased cardiac output then disturbs  $\dot{V}_A/\dot{Q}$  relationships in the lung leading to a further fall in  $P_{aO_2}$ . Our results indicate, however, that hypoventilation was not present with barbiturate anesthesia, since no elevation of  $P_{aCO_2}$  or depression of  $P_{aH}$  was seen. For these reasons, decreased lung perfusion due to pentothal is probably the cause of the difference between anesthetized and unanesthetized post-operative arterial blood gas levels.

Our findings are in keeping with those of Yeh et al (125), with respect to the arterial pH and  $P_{aCO_2}$ , who, however, found abnormal oxygenation to be a constant feature in dogs subjected to unilateral immediate autotransplantation. In their study, light nembutal anesthesia with no respiratory assistance was used for pulmonary function studies. Following autotransplantation, the mean  $P_{aO_2}$  fell 11 mm.Hg, and (A-a)  $O_2$  increased 14 mm.Hg. These changes were attributed to diffusion and perfusion defects since venous admixture was increased, and carbon monoxide uptake decreased in the operated group. Although ventilation was reported to be unchanged, in view of our findings, we feel that the changes reported by Yeh et al.,



were due to nembutal anesthesia rather than the effects of autotransplantation.

4. (a-A) CO<sub>2</sub> (ARTERIO-ALVEOLAR CO<sub>2</sub> GRADIENT) and (A-a) O<sub>2</sub> (ALVEOLAR-ARTERIAL O<sub>2</sub> GRADIENT).

(a-A) CO<sub>2</sub> results are summarized in Table 6. No significant gradient was found in any of the groups studied. Similar results were obtained for (A-a) O<sub>2</sub>, i.e., no significant gradient was observed in any group studied (Table 7).

The basic idea of the  $\dot{V}_A/\dot{Q}$  concept is that uneven distribution of ventilation and/or blood flow to various portions of the lung normally leads to an alveolar-arterial difference for O<sub>2</sub>, but not necessarily for CO<sub>2</sub>. Normally, over-ventilation of some parts of the lung (with respect to their perfusion) washes out CO<sub>2</sub> to the same extent that under-ventilation of other parts fails to wash it out, producing a normal arterial CO<sub>2</sub> content. That is, (a-A) CO<sub>2</sub> is small. However, the same is not true for O<sub>2</sub>, since over-ventilation of a portion of the lung cannot increase the P<sub>O<sub>2</sub></sub> of the blood passing through it, due to the shape of the hemoglobin-oxygen dissociation curve. For this reason, as well as the decreased diffusion of O<sub>2</sub> across the alveolar membrane as compared with CO<sub>2</sub>, the (A-a) O<sub>2</sub> is normally large. From these facts has arisen the concept of ventilation ( $\dot{V}_A$ ), perfusion ( $\dot{Q}$ ) relationship or  $\dot{V}_A/\dot{Q}$  (227). Any inequality of  $\dot{V}_A/\dot{Q}$  must lead to a P<sub>aCO<sub>2</sub></sub> higher than the P<sub>ACO<sub>2</sub></sub> (mixed alveolar gas), but because of the great diffusing capacity of CO<sub>2</sub> and the small arterio-venous P<sub>CO<sub>2</sub></sub> differ-





TABLE 6

ARTERIAL-ALVEOLAR CO<sub>2</sub> GRADIENTS

Group	Dog No.	F <sub>A</sub> CO <sub>2</sub> (%)	P <sub>A</sub> CO <sub>2</sub> (mm. Hg)	P <sub>a</sub> CO <sub>2</sub> (mm. Hg)	a-A CO <sub>2</sub> gradient (mm. Hg)
<u>Control</u>					
	495	5.9	37.4	38.0	+0.6***
	499	8.6	57.0	54.0	-3.0
	505	6.0	42.0	40.0	-2.0
	473	4.8	31.0	31.0	nil
	496	6.5	42.3	42.0	-0.3
	1011*	5.4	36.0	36.0	nil
		4.9	32.8	33.5	+0.7
		4.6	30.3	31.0	+0.7
		5.0	33.0	33.0	nil
	<u>x</u>	5.75	37.94	37.61	-0.33
			<u>+5.60**</u>	<u>+4.84</u>	
<u>Immediate Reimplantation</u>					
	373	6.1	39.7	40.0	+0.3
	440	6.9	45.0	45.5	+0.5
	356	6.5	42.3	42.5	+0.2
	411	6.0	39.0	38.5	-0.5
	442	6.5	42.2	42.0	-0.2
	434	5.4	35.1	35.5	+0.4
	383	6.1	39.7	41.0	+1.3
	<u>x</u>	6.21	40.40	40.71	+0.285
			<u>+2.36</u>	<u>+2.4</u>	<u>+ .430</u>
<u>Delayed Reimplantation</u>					
4 hour	797	5.0	27.5	28.0	+0.5
	873	6.5	42.0	44.2	+2.2
	798	6.6	39.0	38.8	-0.2

.....continued.....

\* Determinations carried out four times in succession on same dog and under same conditions.

\*\* S<sub>x</sub> at 95% level.

\*\*\* += P<sub>A</sub>CO<sub>2</sub> < P<sub>a</sub>CO<sub>2</sub> could be physiologic gradient.

- = P<sub>A</sub>CO<sub>2</sub> > P<sub>a</sub>CO<sub>2</sub> therefore calibration error.





TABLE 6 (Cont.)

Group	Dog No.	F <sub>A</sub> CO <sub>2</sub> (mm. Hg)	P <sub>A</sub> CO <sub>2</sub> (mm. Hg)	P <sub>a</sub> CO <sub>2</sub> (mm. Hg)	a-A CO <sub>2</sub> gradient (mm. Hg)
<u>Delayed reimplantation</u> (cont.)					
4 hour	861	5.2	33.0	33.9	+0.9
	968	6.6	38.0	37.5	-0.5
	596	7.1	42.0	41.7	-0.3
	<u>853</u>	<u>5.3</u>	<u>35.5</u>	<u>35.2</u>	<u>-0.3</u>
6 hour	<u>612</u>	<u>7.1</u>	<u>42.5</u>	<u>42.0</u>	<u>-0.5</u>
	x	6.17	37.44	37.66	-0.287
			<u>+3.70</u>	<u>+3.70</u>	<u>+0.22</u>
<u>Delayed reimplantation</u>					
6 hour (HPO)	1122	7.1	42.3	42.0	+0.3



TABLE 7

ALVEOLO-ARTERIAL OXYGEN GRADIENTS

$$(A-a) O_2 (FI_{O_2} = 0.2009)$$

Group	Dog No.	$FE_{O_2}$	$FA_{CO_2}$	$FE_{CO_2}$	$FA_{O_2}$	$PA_{O_2}$	$Pa_{O_2}$ (mm. Hg)	$(A-a)O_2$ (mm. Hg)
<u>Control (3% chloralose Anesthesia)</u>								
	1	.1684	.0515	.0240	.1329	87.71	74	13.71
	2	.1730	.0545	.0275	.1457	96.20	64	32.2
	3	.1715	.0460	.0225	.1400	92.40	65	27.4
	4	.1988	.0410	.006	.1866	123.0	98	25.0
	5	.1797	.0720	.042	.1761	116.23	94.5	11.73
	6	.1780	.0420	.011	.1134	74.84	70	4.84
	x	.1855	.0517	.0197	.1494	98.39	77.58	19.90
<u>Operated (3% chloralose Anesthesia)</u>								
	873	.1567	.060	.0350	.1253	82.70	64	18.70
	repeat	.1473	.060	.044	.1249	82.06	74	8.06
	798	.1704	.054	.028	.1345	87.29	80.3	6.99
	797	.1650	.0595	.0305	.1227	79.63	76	3.63
	499	.1506	.0735	.042	.1132	74.71	59	15.71
	612	.1817	.0630	.0185	.1358	89.63	71	18.63
	861	.1679	.0460	.0267	.1441	95.11	73.5	21.61
	440	.1727	.0600	.0295	.1437	94.84	77	17.84
	repeat	.1658	.061	.030	.1411	91.57	72	19.57
	repeat	.1596	.063	.035	.1197	77.69	67	10.69
	798	.1764	.054	.028	.1345	87.29	80.3	6.99
	356	.1808	.073	.017	.0864	56.76	54	2.76
	442	.1595	.060	.036	.1250	82.78	75	7.78
	1122	.1648	.046	.035	.1509	99.14	96	3.14
	968	.1344	.084	.055	.1149	75.49	51	24.49
	repeat	.1344	.076	.046	.0841	55.25	51	4.25
	853	.1511	.066	.0465	.1266	83.18	65	18.18
	repeat	.1420	.066	.0465	.1136	74.64	65	9.64
	repeat	.1460	.066	.0465	.1202	78.97	65	13.97
	x	.1590	.066	.0356	.1243	81.51	69.26	12.24





ence, a large impairment of pulmonary function must exist before a significant (a-A) CO<sub>2</sub> becomes apparent. For example, a pulmonary arteriovenous shunt of 10% of the cardiac output would produce only a 0.5 mm.Hg (a-A) CO<sub>2</sub> (assuming normal venous PCO<sub>2</sub>)(228).

The experimental error for the method used in our study is probably at least 1.0 mm.Hg PCO<sub>2</sub>. Also, determination of the mean P<sub>A</sub>CO<sub>2</sub> is not easy. Ulmer and Reichel (229) compared P<sub>A</sub>CO<sub>2</sub> at various times during expiration to the P<sub>a</sub>CO<sub>2</sub>. During normal expiration, the P<sub>A</sub>CO<sub>2</sub> plateau begins below the P<sub>a</sub>CO<sub>2</sub> and rises above it by the end of expiration. This rising P<sub>A</sub>CO<sub>2</sub> plateau was also seen in the present study (Fig.29). Thus, if P<sub>A</sub>CO<sub>2</sub> values are selected from early expiration, (a-A) CO<sub>2</sub> is positive (i.e. P<sub>A</sub>CO<sub>2</sub> < P<sub>a</sub>CO<sub>2</sub>); if selected from late expiration (a-A) CO<sub>2</sub> becomes negative (P<sub>A</sub>CO<sub>2</sub> > P<sub>a</sub>CO<sub>2</sub>). Therefore, it is conceivable that as much as a 20% of the cardiac output could be perfusing unventilated portions of the autotransplanted lung, and remain undetected by the present method. Yeh et al. (125), found an increase in pulmonary arteriovenous shunting from 4 to 9% of cardiac output following unilateral autotransplantation. In our study the absence of increased (A-a) O<sub>2</sub> and normal oxygen uptake by the autotransplanted lung (see infra) indicates that no such shunt exists. Again, this inconsistency in results could be due to Yeh et al's use of nembutal anesthesia for pulmonary function studies.



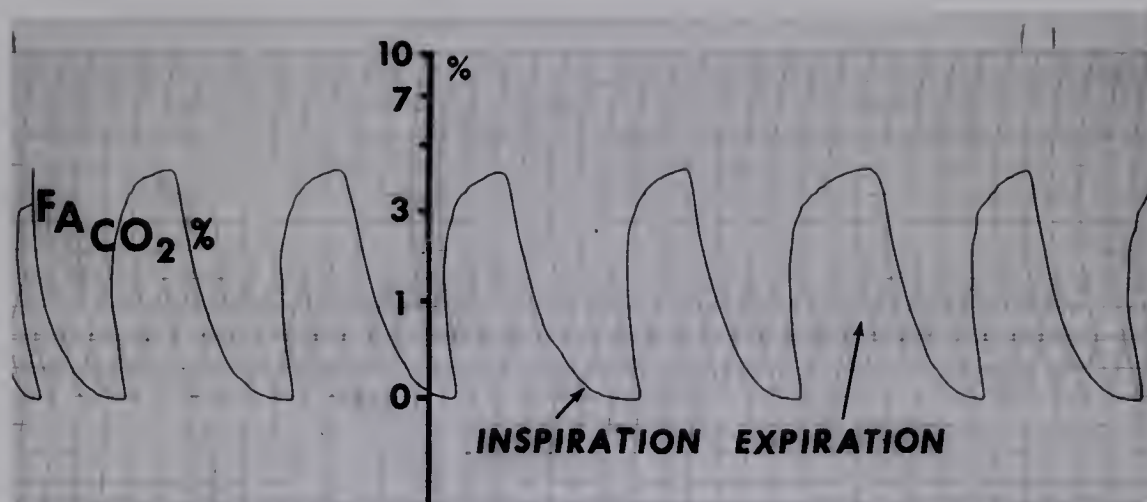


Figure 29. Tracing of Inspired and Expired  $F_{\text{CO}_2}$  following immediate reimplantation.





## 5. DIFFERENTIAL BRONCHOSPIROMETRY.

Differential bronchspirometry using a double lumen endotracheal tube offers a method of obtaining a quantitative measure of the function of the two lungs separately and simultaneously. Studies of normal human subjects show that the right lung is responsible for 55% of the total pulmonary ventilation and oxygen consumption, while the left lung contributes 45% of each (230). Any marked deviation from these ratios suggests unilateral pulmonary disease. For example, if the right lung contributed 55% of total ventilation but none of the oxygen uptake, a severe diffusion defect or the absence of blood flow through that lung might be present.

Measurement of differential ventilation will detect uneven distribution of inspired gases, but differential oxygen uptake measurement is necessary to assess pulmonary capillary perfusion and the diffusion properties of the alveolar membrane.

In the present study, when care was taken to avoid occlusion of the upper lobe bronchi and when the White tube was used, ventilation and oxygen consumption for the respective lungs correlated well both with regard to each other and with expected normal values (Table 8, Figs. 30,31,32,33). In the control group a Carlens catheter was used. Oxygen consumption by the left lung was less than the left lung ventilation. Control oxygen uptake was also less than that observed in the experimental groups, for which a White tube was used. This difference might be accounted for by the fact that the Carlens catheter





TABLE 8

DIFFERENTIAL BRONCHOSPIROMETRY - CONTROL AND OPERATED DOGS

Group	Dog No.	Left Lung %O <sub>2</sub> uptake	Left Lung % vent.	Right Lung %O <sub>2</sub> uptake	Right Lung % vent.	Hering-Breuer Reflex (+=present -=absent) Left Lung	Right Lung	Time Post Operative
Control	1	49	49	51	52	+	+	
	2	36	43	64	57	+	+	
	3	32	46	68	54	+	+	
	4	50	48	50	52	+	+	
	5	46	48	54	52	+	+	
	x	42.60	46.60	57.40	53.40			
Immediate Reimplant- ation	373	48.5	45.0	51.5	55.0	-	+	7 mo.
	440	52.5	50.0	47.5	50.0	-	+	6 mo.
	356	58.0	51.0	42.0	49.0	-	+	7 mo.
	442	48.0	40.0	62.0	60.0	+	+	5 1/2 mo.
	434	62.0	75.0	38.0	25.0**	-	+	11 mo.
	499	46.0	50.0	54.0	50.0	-	+	4 1/2 mo.
	x	48.6	47.2	51.4	52.8			
Delayed Reimplant- ation	797	46.0	50.0	54.0	50.0			1 month
	873	40.0	32.0	60.0	68.0	+	+	1 month
	798	48.0	50.0*	52.0	50.0*	-	+	1 month
	861	48.5	39.0	51.5	61.0	-	+	1 month
	968	54.5	47.0	45.5	53.0	+	+	1 month

.....continued.....

\* Tidal volume small, therefore ventilation difficult to assess but grossly equal.

\*\* Right upper lobe bronchus probably occluded, therefore not included in statistical analysis.



Table 8 (cont.) - Differential Bronchspirometry.

Group	Dog No.	Left Lung %O <sub>2</sub> uptake vent.	Right Lung %O <sub>2</sub> uptake vent.	Hering-Breuer Reflex (+=present -=absent)	Time Post Operative
(Cont.)					
Delayed Reimplantation					
4 hour hypothermia	596	41.0	59.0	-	1 month
	853	22.5	77.5	-	1 month
	x	42.93	57.07		
		43.71	56.29		
6 hour hypothermia	612	48.5	51.5	-	1 month
		46.0	54.0	+	
6 hour hypothermia + HPO (3 atmos.)	1122	43.0	57.0	-	3 weeks
		54.0	46.0	+	

\*\*\*Repeated on three occasions 2 weeks apart.





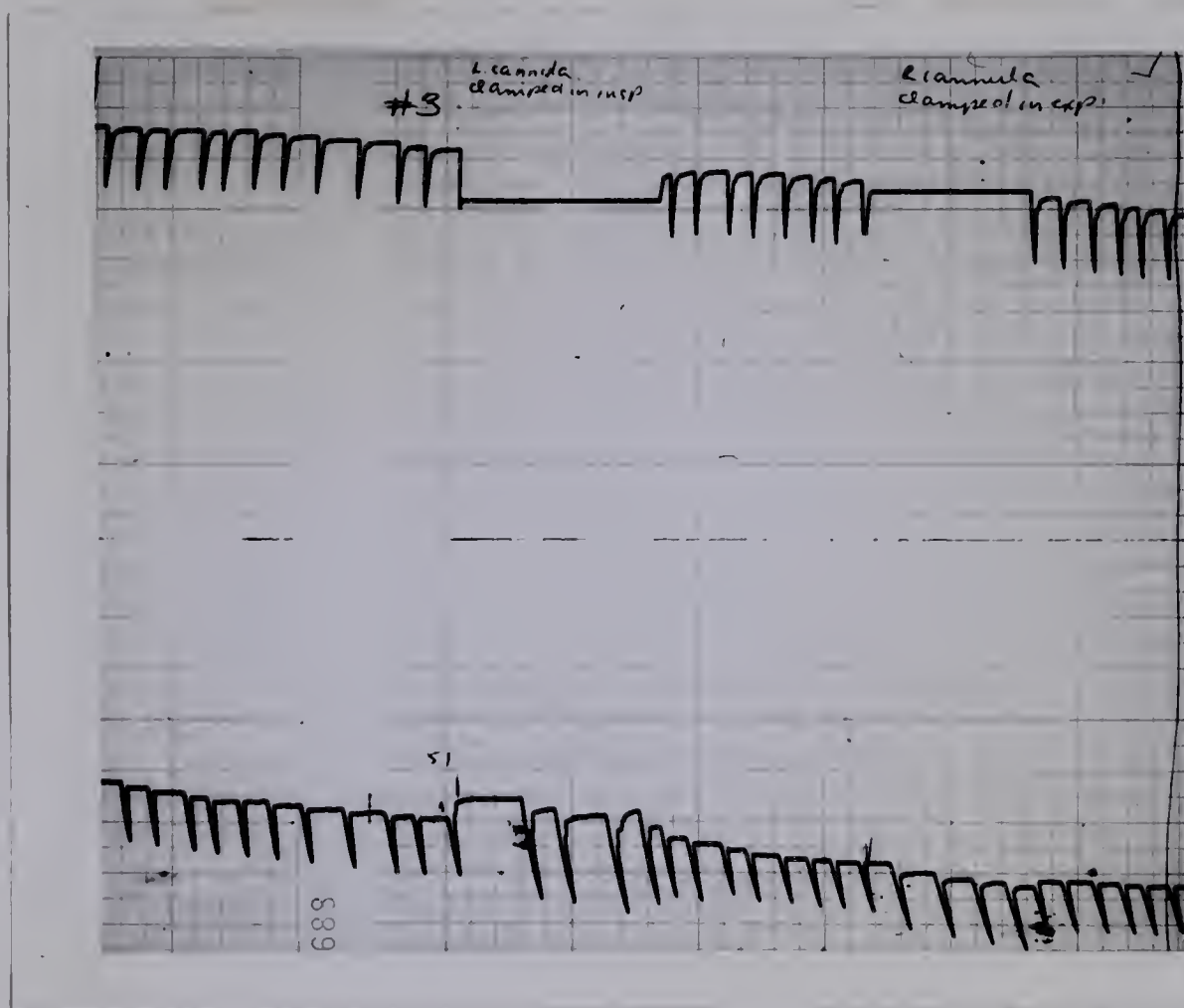


Figure 30. Differential Bronchspirometry Control. Tracings from left lung above, right lung below. Note Hering-Breuer response in right lung ventilation following occlusion of left lung cannula in full inspiration.



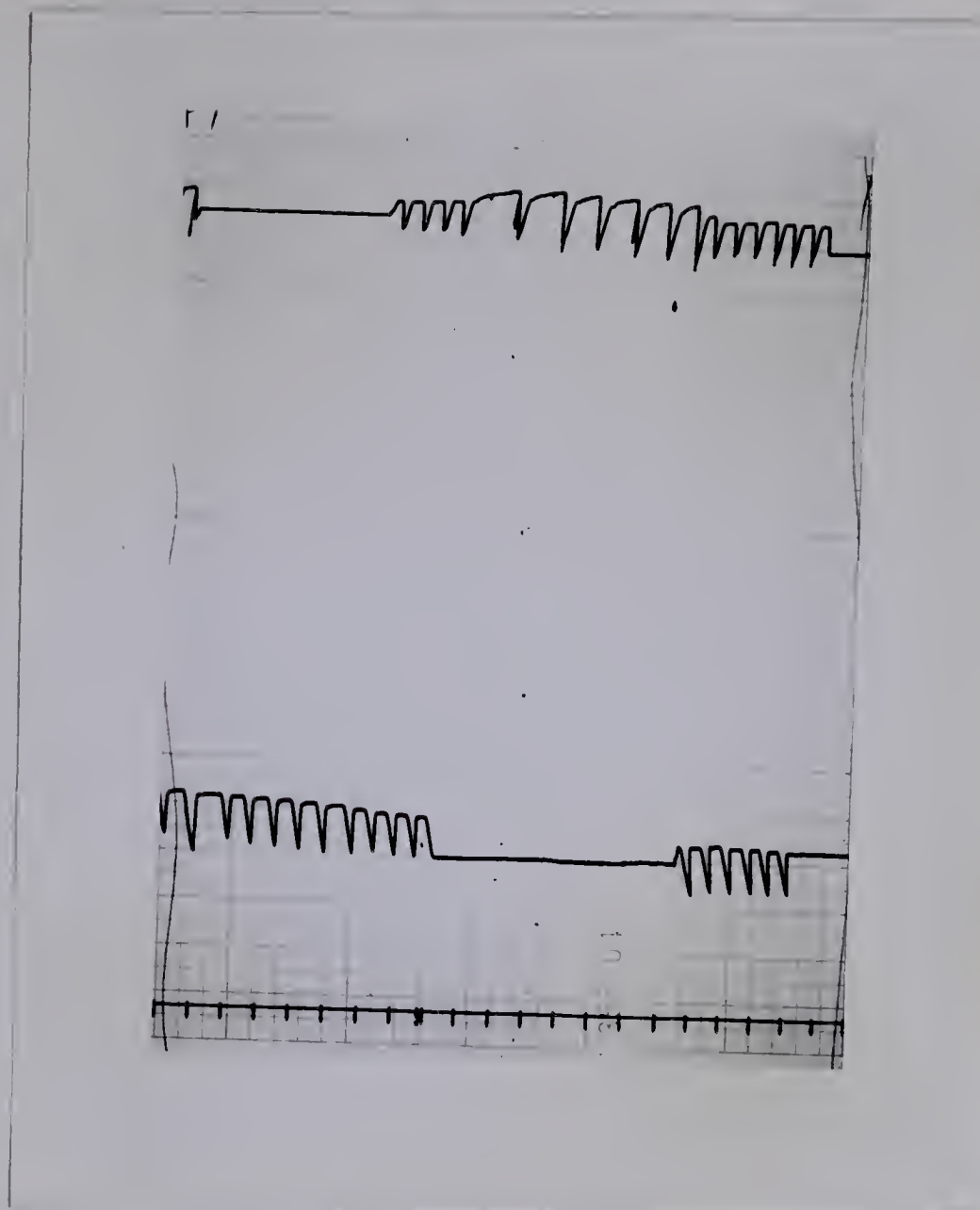


Figure 31. Differential Bronchspirometry Tracing After Immediate Reimplantation. Upper tracing left lung ventilation, lower, right lung. Left lung cannula occluded in deep inspiration. No inhibition of right lung inspiration (H-B reflex absent). Right lung cannula occlusion produces inhibition of right lung inspiration (H-B reflex present).



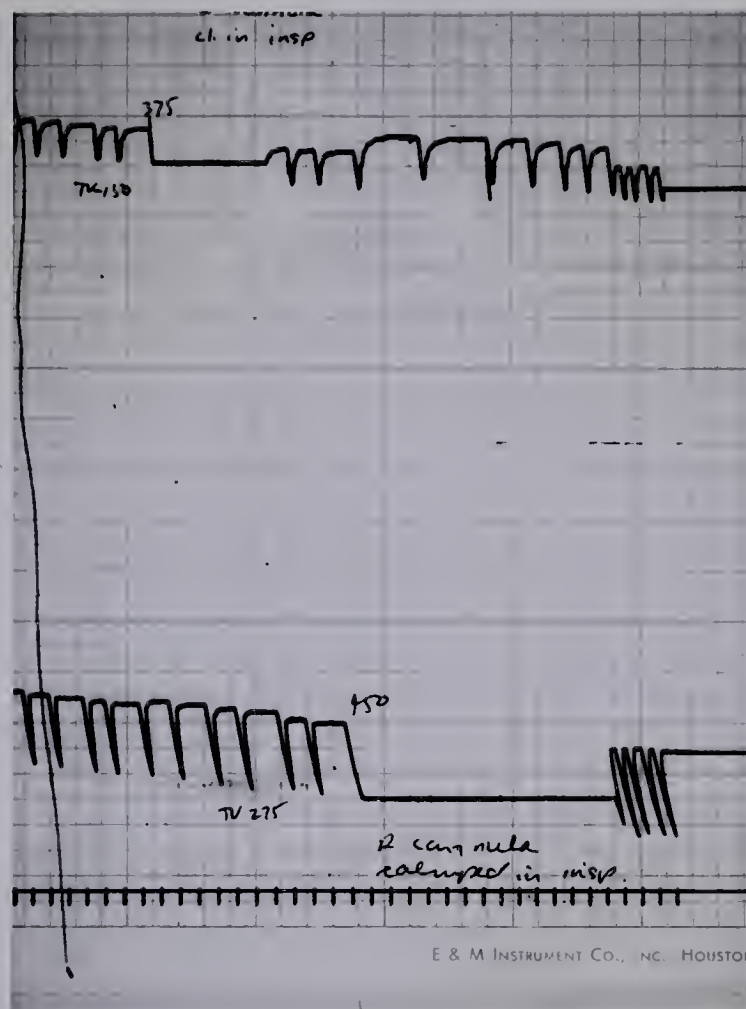


Figure 32. Differential Bronchspirometry Following Immediate Reimplantation. Left lung tracing above, right below. H-B reflex absent. When left lung maintained in inflation, right H-B is present.





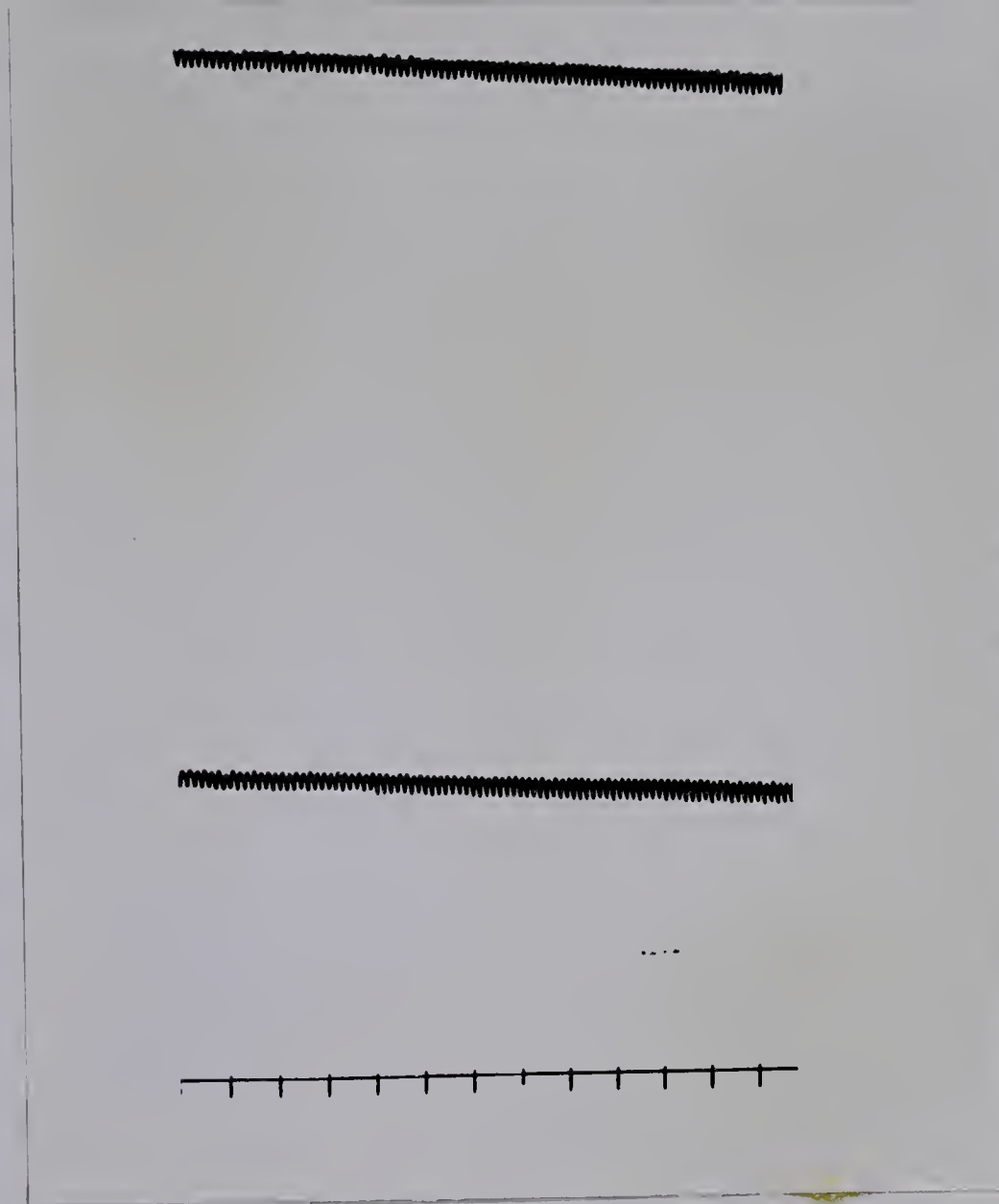


Figure 33. Differential Bronchspirometry Following Four Hour Hypothermic Lung Preservation and Reimplantation. Left lung tracing above. Time marker interval five seconds. Note smooth  $O_2$  uptake and equal ventilation.



intubates the left mainstem bronchus; inflation of the cuff within the bronchus might lead to partial occlusion of the left pulmonary artery, passing superiorly in intimate contact with the bronchus. This would, in turn, lead to decreased perfusion and left lung oxygen uptake. In any event, no diminution in either ventilation or oxygen uptake by the left lung was observed, following immediate or delayed reimplantation of hypothermic lungs. Since spirometry was not performed until one to six months following operation, it is probably that considerable return of function had occurred by the time the tests were performed. Three operated dogs showed what appeared to be the presence of the left Hering-Breuer reflex as little as one month post-transplant; i.e., following occlusion of the cannula from the right lung in inspiration, left lung inspiration was inhibited. It is difficult to see how the Hering-Breuer reflex could return within one month of total lung denervation. However, proprioceptive reflexes from sensory (possibly muscle spindle) endings in the chest wall also inhibit inspiration when the chest wall is maintained expanded (223). This reflex might be responsible for the above experimental findings.

#### 6. RIGHT HEART CATHETERIZATION.

No statistical difference was found between mean pulmonary artery pressures obtained from control, immediate and delayed reimplantation groups (Table 9). As mentioned previously (page 21), Nigro's work indicates that a state of vasospasm





TABLE 9

RIGHT HEART CATHETERIZATION PRESSURES-CONTROL AND OPERATED DOGS

Group	Dog or Exp. No.	Pulmonary Artery			Right Ventricle		
		S	D	M	S	D	M
Control	1	20.0	15.0	17.5			
	2	23.0	16.5	14.0			
	3	27.0	19.5	22.5			
	4	35.1	19.5	26.0			
	5	24.7	14.3	19.5			
	6	16.0	9.0	13.0			
	7	18.5	10.5	14.5			
	8	28.0	15.0	19.0			
	9	21.0	10.5	16.0			
	10	24.0	8.0	17.0			
	11	20.0	7.0	13.0			
	x	24.23	13.16	17.45 +2.54			
Immediate Reimplantation							
	434	31.0	20.0	25.0	-	-	-
	440	20.0	18.0	19.0	21.0	8.0	13.0
	356	26.0	15.5	19.0	37.0	6.5	16.6
	373	26.0	8.0	14.0	36.0	0	12.0
	499	25.0	29.0	23.75	26.0	11.0	20.0
	442	18.0	12.4	15.0	19.0	8.0	13.0
	411	23.0	6.0	11.6	-	-	-
	x	24.15	14.27	18.19 +3.76	27.80	6.70	14.92
Delayed Reimplantation							
4 hour hypo- thermia	596	25.0	20.0	23.0	26.75	11.0	17.5
	798	29.0	17.5	21.0	38.0	0	18.5
	797	36.0	23.0	28.0	40.0	0	28.0
	968	26.0	23.0	24.0	34.0	6.0	17.0
	873	19.0	11.0	15.5	24.0	7.0	16.0
	853	16.2	10.0	13.0	20.0	11.2	13.3
	861	25.0	20.0	22.0	23.75	12.5	17.0
	x	25.17	17.79	20.93 +3.83	30.93	6.81	18.19
6 hour hypothermia	612	25.0	17.0	19.6	27.0	4.0	8.0
6 hour hypothermia + HPO	1122	26.0	15.0	19.0			



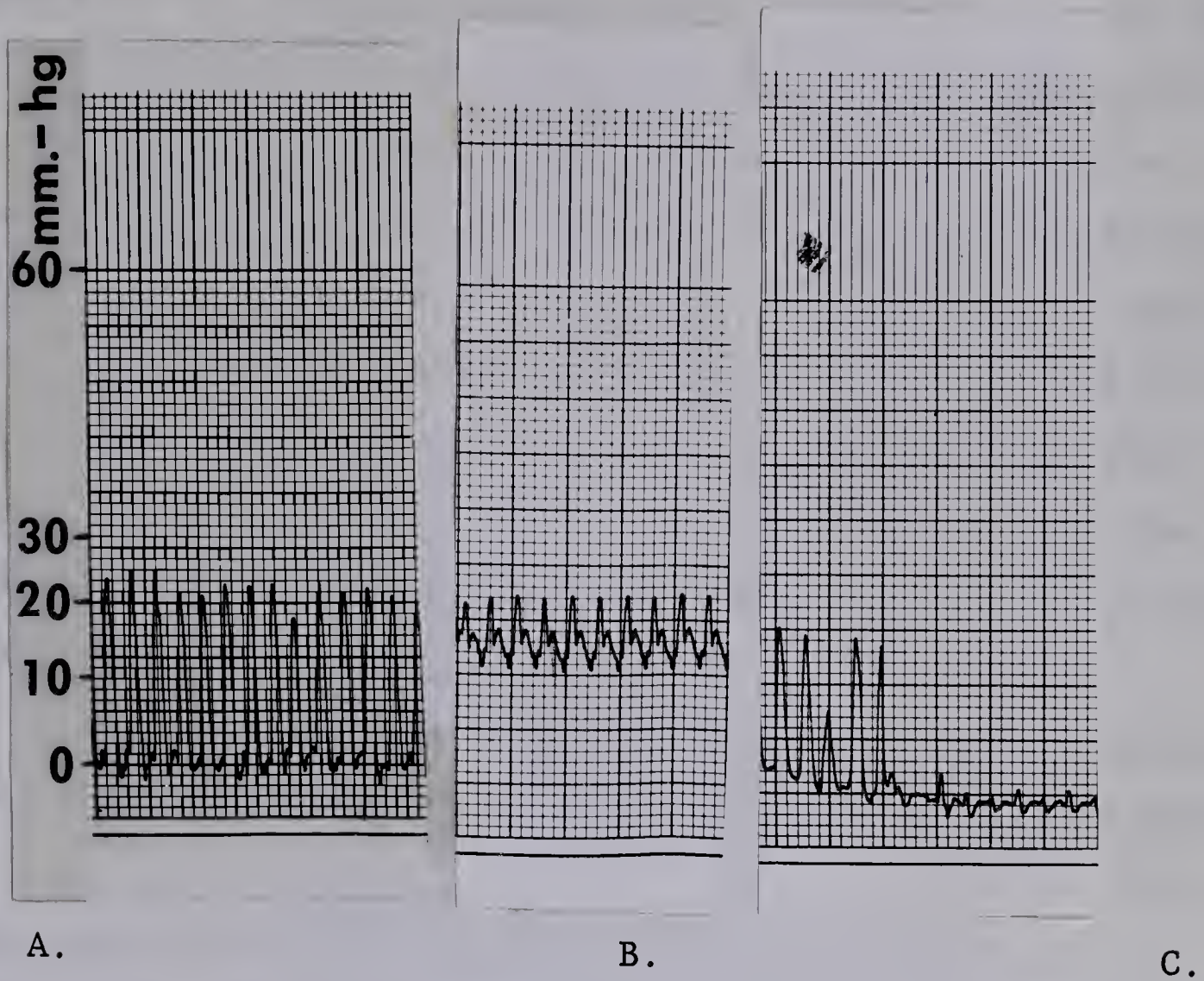


Figure 34. Right Heart Catheterization Pressures. Dog No. 612. Left Lung Subjected to Six Hours Hypothermia Prior to Reimplantation. A, B, C, - Right Ventricular, Pulmonary Artery and Right Atrial Pressures Respectively.





exists in the autotransplanted lung. If this were true, normal pulmonary artery pressures could only be maintained if blood flow through the normal right lung was increased. In our series (A-a)  $O_2$ , (a-A)  $CO_2$  and differential bronchspirometry studies do not confirm this finding. It is therefore probable that blood flow through the graft under the experimental conditions imposed is normal. However, it is possible that the superimposed stress of contralateral pneumonectomy on the graft vasculature might show up the graft's inability to withstand a major increase in blood flow.

Mainly responsible for the idea that the autotransplanted lung is a "damaged" one, is the work of Drs. Hardy and Reemtsma. In fact, other investigators have found little or no functional impairment of any kind following lung autotransplantation. In addition, Hardy and Reemtsma's work is open to criticism since their conclusions depend chiefly on measurement of oxygen uptake. On close scrutiny of their results, one finds that many of the normal values reported for oxygen uptake by the left lung are well above 50% of the total. Since the left lung contributes only 40 to 45% of the total lung volume, it is difficult to see how the left lung  $O_2$  uptake could exceed that of the right lung. In any case, even if the lung transplant has impaired function, it still participates in respiration to a considerable degree. When one subjects a patient to gastrectomy, one does not expect him to have completely normal, but only adequate gastric function for his needs. A similar attitude toward lung transplantation





might be adopted, with considerable benefit accruing to the patient suffering from intractable respiratory disease.



Figure 35. Thoracic Contents of Dog Subjected to Four Hour Left Lung Hypothermia and Reimplantation. Note absence of pleural adhesions and broncho-stenosis. (Operated lung on left).





Figure 36. Bronchial Anastomosis Three Months Following Immediate Reimplantation







Figure 37. Left Pulmonary Artery Anastomosis Six Weeks Following Hypothermic HPO Preservation and Reimplantation.



Figure 38. Left Atrial Anastomosis Three Months Following Four Hour Hypothermic Preservation and Reimplantation.





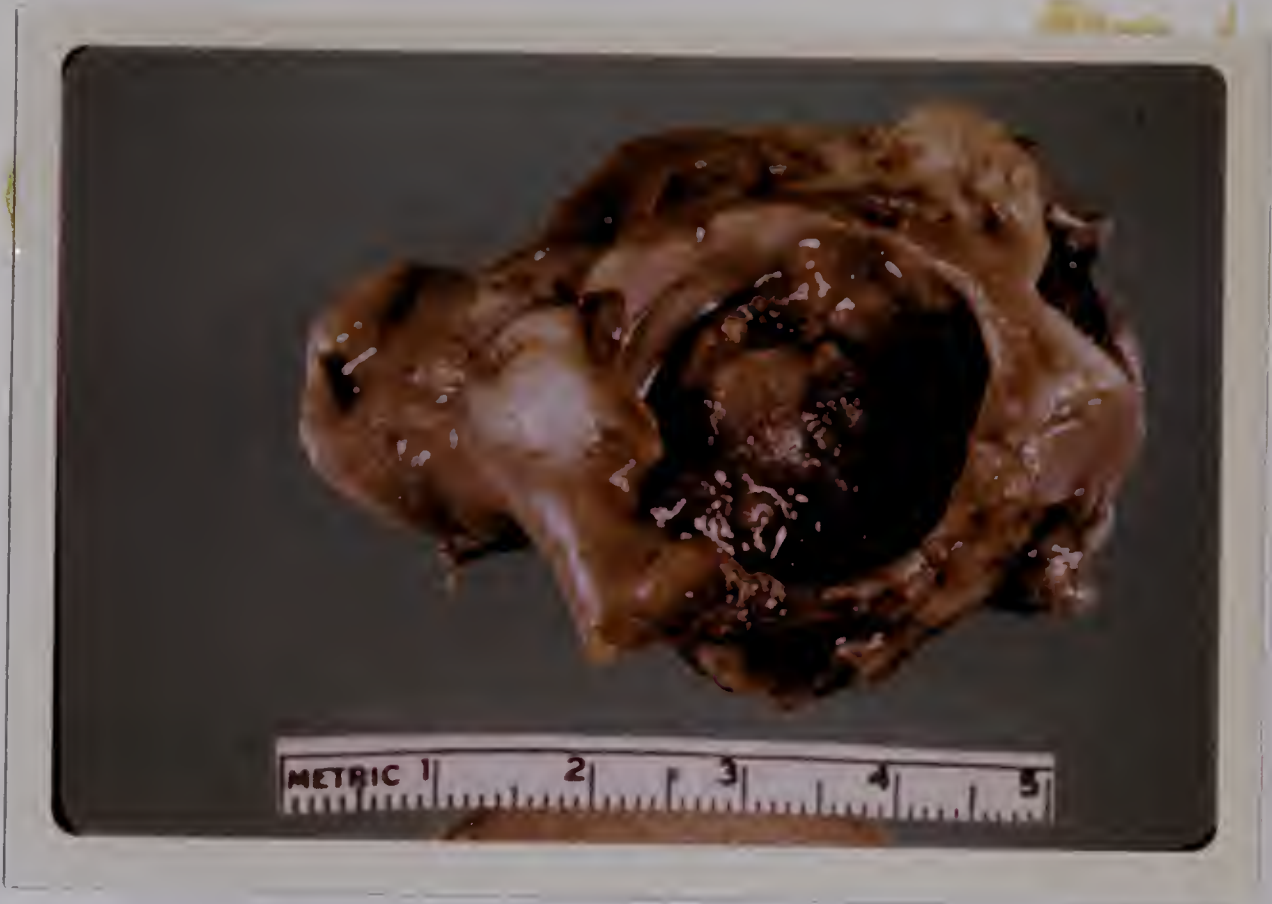


Figure 39. Left Atrial Thrombus Four Days Following Six Hour Hypothermic Preservation and Reimplantation.



Figure 40. Left Atrium Six Weeks Following Six Hour HPO Hypothermic Lung Preservation and Autotransplantation. Note recannulating thrombus over orifices of left pulmonary veins.





Figure 41. Tracheal Carina Six Weeks Following Six Hour HPO Hypothermic Lung Preservation and Auto-transplantation. Stenosis of left mainstem bronchus at suture line.





1. The first of these is the  
the second is the  
the third is the  
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the fifth is the

(1000)

ISOLATED LUNG EXPERIMENT A.

Under the imposed conditions of the experiment, the perfused and ventilated lungs invariably gained weight. Pulmonary edema, evaluated by direct observation during perfusions, began to develop within 20-30 minutes of commencement of perfusion despite perfusion pressures of 20 mm.Hg or less. At the end of three hours of perfusion, gross edema was apparent (Fig. 42). Post-perfusion weights were approximately double pre-perfusion weights (Table 10, Fig. 52).

In the initial series of experiments, the lungs were ventilated with non-humidified 97% O<sub>2</sub> air mixture. The pH of the perfusate within 30 minutes rose to >8.0 and remained too high for measurement for the remainder of the perfusion. Attempts to buffer the plasma with NH<sub>4</sub>Cl failed. At the same time as the perfusate pH rose, perfusate PCO<sub>2</sub> dropped to virtually unmeasurable levels. It was thought that the addition of CO<sub>2</sub> to the inspired air might offset these effects. Therefore in three perfusion experiments, various levels of inspired CO<sub>2</sub> were administered in an attempt to control the plasma pH. It was found that the plasma pH did indeed change with PICO<sub>2</sub>, and that the relationship of plasma PCO<sub>2</sub> to pH was a logarithmic one, as is seen normally (Figs. 43,44). The plasma pH and PCO<sub>2</sub> varied markedly with perfusate temperature, as reported by Severinghaus et al., who state that pH changes 0.0146 units per degree temperature change. PCO<sub>2</sub> and PO<sub>2</sub> change approx-



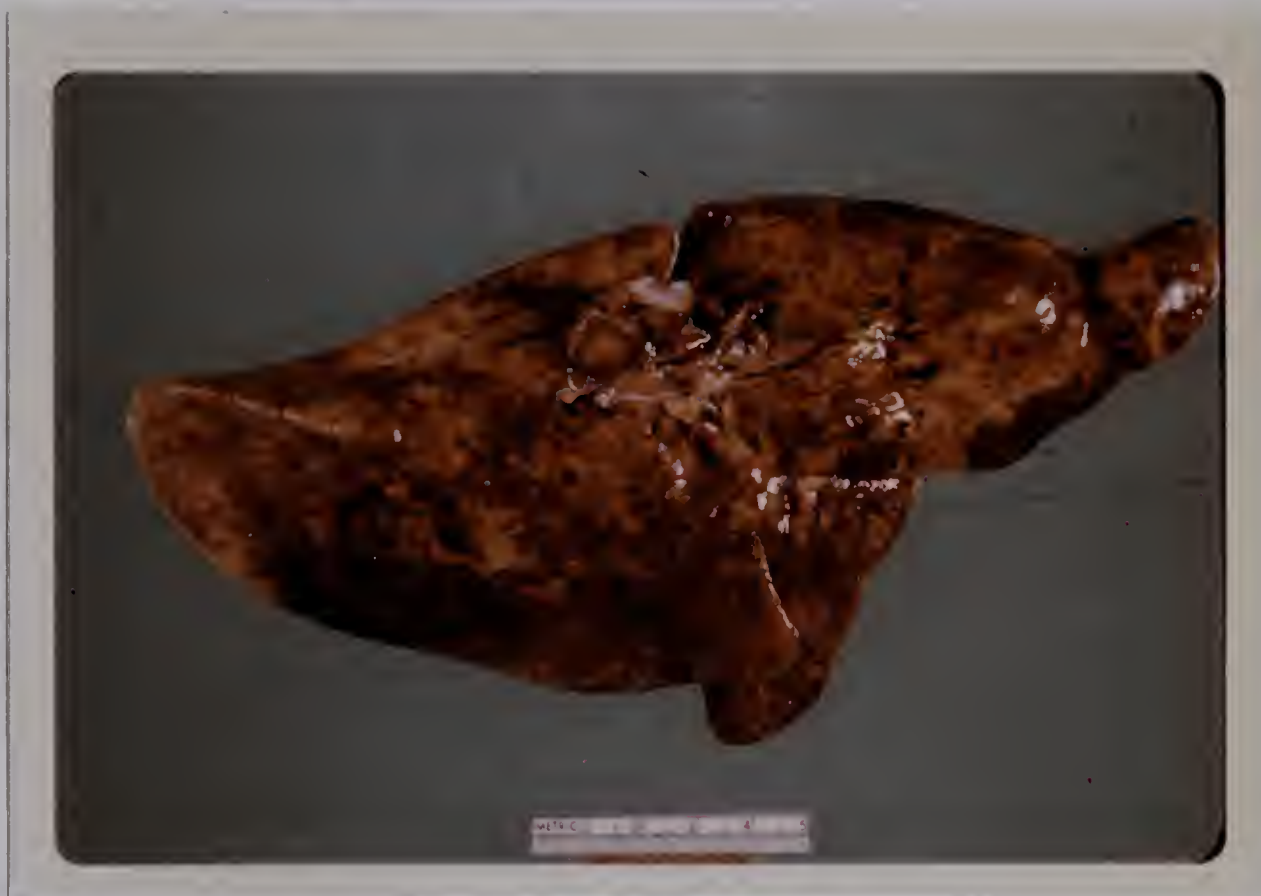


Figure 42. Isolated Lung Experiment B. Although edema is generalized, most occurs below a line drawn through the most dependent portions.





TABLE 10

ISOLATED LUNG EXPERIMENTS A. AND B.

Pre- and Post-Perfusion Lung Weights

Group	Exp. No.	Pre-Perf. Lung Wt. (gms)	Post-Perf. Lung Wt. (gms)	Wt Gain (gms)	% Increase
Isolated Lung Experiment A.	1	61.5	125.0	63.5	103.20
	2	44.0	90.0	46.0	104.54
	3	54.5	112.0	57.5	105.50
	4	62.0	170.0	108.0	174.18
	5	56.0	94.0	28.0	50.00
	6	50.0	120.0	70.0	104.00
	7	40.0	-	-	-
	8	52.0	-	-	-
	x	54.3	118.50	64.2	110.86
Isolated Lung Experiment B	1	52.5	180.0	127.5	242.9
	2	62.0	80.0	18.5	29.8
	3	40.0	88.1	48.9	120.5
	4	60.0	114.0	54.0	190.0
	5	32.0	90.5	58.0	
	6	44.25	97.25	53.0	181.25
	7	33.0	107.50	74.9	127.0
	8	57.0	81.0	24.0	42.11
	9	50.0	82.0	32.0	64.0
	x	47.64	102.30	65.64	110.84



# ISOLATED LUNG EXP. A.

RELATIONSHIP of PLASMA  $P_{CO_2}$  to INSPIRED  $CO_2$  LEVEL

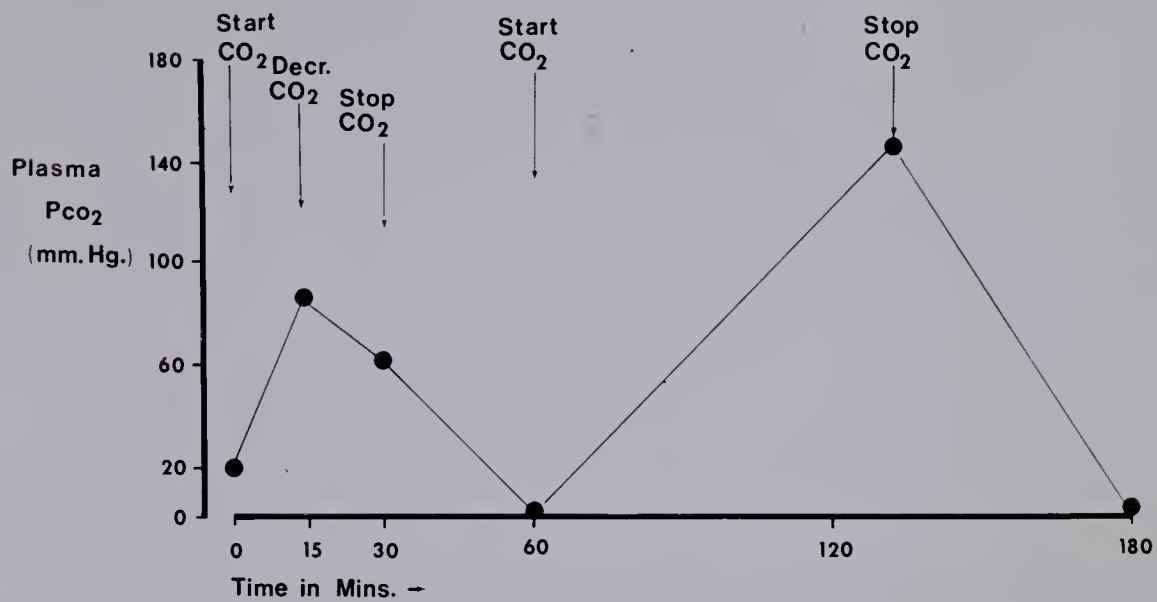


Figure 43. Isolated Lung Experiment A. -  $P_{aCO_2}$  v.s.  $P_{ACO_2}$

# ISOLATED LUNG EXPERIMENTS

RELATIONSHIP of LOG. PLASMA  $P_{CO_2}$  to PLASMA pH

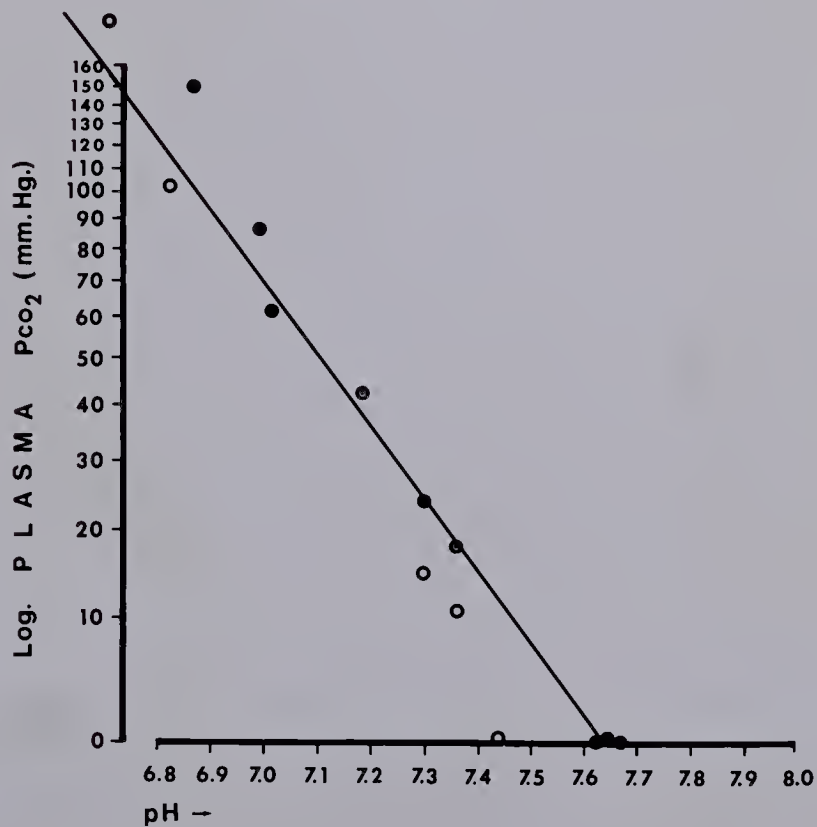


Figure 44. Isolated Lung Experiment A. - Log  $P_{aCO_2}$  v.s.  $P_{aH}$



imately 4.4 and 6% respectively per  $^{\circ}\text{C}$  temperature change (182).

It is unclear which pH,  $\text{P}_{\text{O}_2}$  and  $\text{P}_{\text{CO}_2}$  would be active physiologically under the imposed conditions (that withdrawn at  $15^{\circ}\text{C}$  and measured at body temperature, or that withdrawn at  $15^{\circ}\text{C}$  and corrected to  $15^{\circ}\text{C}$ ). Empirically it was decided to attempt to produce a normal pH taken at  $15^{\circ}\text{C}$  and measured at  $35^{\circ}\text{C}$  and corrected back to  $15^{\circ}\text{C}$ . For these reasons in all subsequent experiments 3%  $\text{CO}_2$  was added to the inspired air.

As various perfusion-ventilation methods were being studied, air embolus was not considered as a cause of lung edema, since it is commonly stated clinically that as much as 1000 cc. of air can be administered intravenously to a patient without adverse effect, provided there is no communication with the systemic circulation other than through the lungs. However, when 1 cc. of air was injected into the arterial line of the isolated lung, perfusion pressure increased markedly (Fig. 45) and pulmonary edema promptly ensued. If perfusion was continued despite the appearance of pulmonary edema, perfusion pressure gradually decreased over a period of about one hour. Howard et al. (231), state that injection of gas into the right side of the circulation in dogs and monkeys results in a rapid increase in pulmonary arterial pressure which reaches a maximum in 30 to 60 seconds, and subsides to normal over the next 12 to 20 minutes. There is a linear relationship





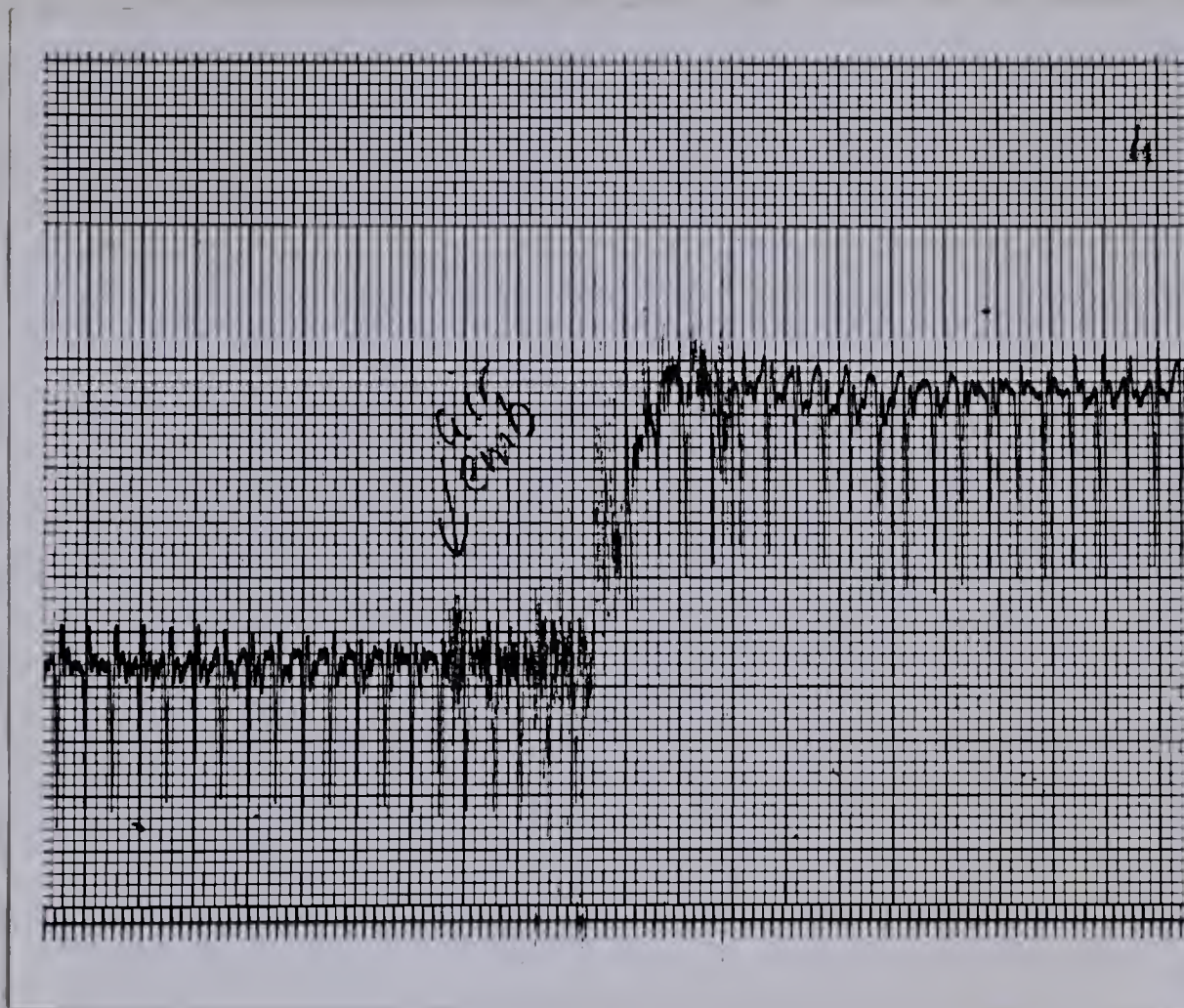


Figure 45. Isolated Lung Experiment A.  
Perfusion pressure tracing  
air embolus.





between volume of gas injected and the magnitude of the hypertension, 1 ml. of air being sufficient to double pulmonary artery mean pressure, with a fall in cardiac output to 70-80% of the control value. Xenon 131 studies showed that none of the injected gas reaches the systemic circulation, but is all excreted via the alveoli. They concluded that the pulmonary hypertension produced by air embolization has a purely mechanical etiology, increased driving pressure being necessary to overcome the increased vascular resistance produced by surface tension forces at blood-gas interfaces.

For these reasons, every effort was made in subsequent studies to prevent air embolization, before, during and subsequent to cannulation of the pulmonary artery for perfusion.

Even after the above problems had been elucidated, a marked increase in all plasma constituents levels occurred when the lung ventilation was unhumidified (Fig.46). Most of the increase took place within the first 15 to 30 minutes. Although it is hard to understand how such a marked degree of perfusate concentration could occur in such a short time by pure evaporation, most of the marked rise in plasma constituents was eliminated by humidification of the inspired gases. This procedure was therefore utilized in all subsequent experiments.

#### Pressure-flow relationships.

During the first few minutes of perfusion, flow was adjusted





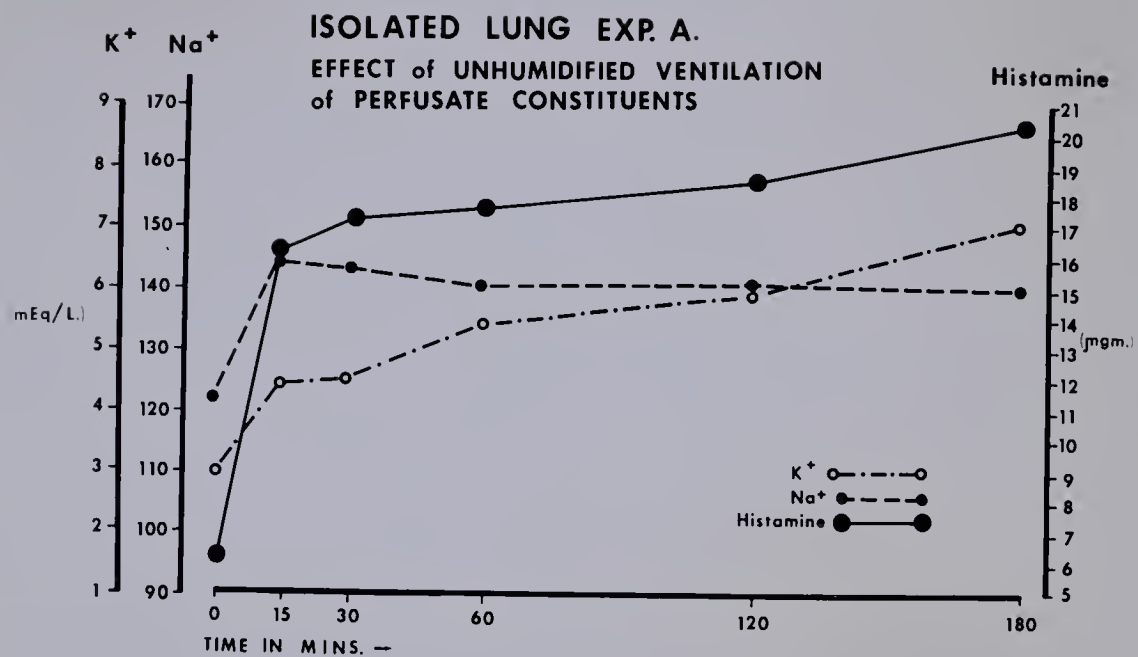


Figure 46. Isolated Lung Experiment A.  
Plasma electrolytes unhumid-  
ified ventilation.



to maintain an approximation of the normal dog pulmonary artery pressure (18-20 mm.Hg). When flow was maintained constant, perfusion pressure fell very gradually until 2 hours perfusion time. By three hours perfusion time mean perfusion pressure had again risen slightly (Addendum Tables 1,2).

Positive pressure inflation markedly influenced perfusion pressure, producing pulmonary artery pressures four times or more higher than pressures obtained when the lung was deflated (Fig. 47).

#### Perfusate gas studies.

Plasma  $PCO_2$  and  $P_{O_2}$  were low and pH high prior to perfusion (see Addendum Tables 3,4,5). Although there are insufficient numbers of observations to draw firm conclusions, it would seem that as perfusion progressed, plasma  $P_{O_2}$  rose in the first 15 minutes to approximately four times the pre-perfusion level and remained relatively constant at a level approximately two-thirds the  $PI_{O_2}$ . Plasma  $PCO_2$  rose and pH fell initially, in response to the added inspired  $CO_2$ .

The calculated  $PI_{CO_2}$  was approximately 20 mm.Hg. At the start of perfusion plasma  $PCO_2$  was slightly higher than  $PI_{CO_2}$  and rose slightly but progressively thereafter, indicating an impairment of  $CO_2$  exchange as pulmonary edema developed. At the same time, plasma pH fell slightly.

#### Perfusate biochemical studies.

Perfusate biochemical studies are summarized in Figures





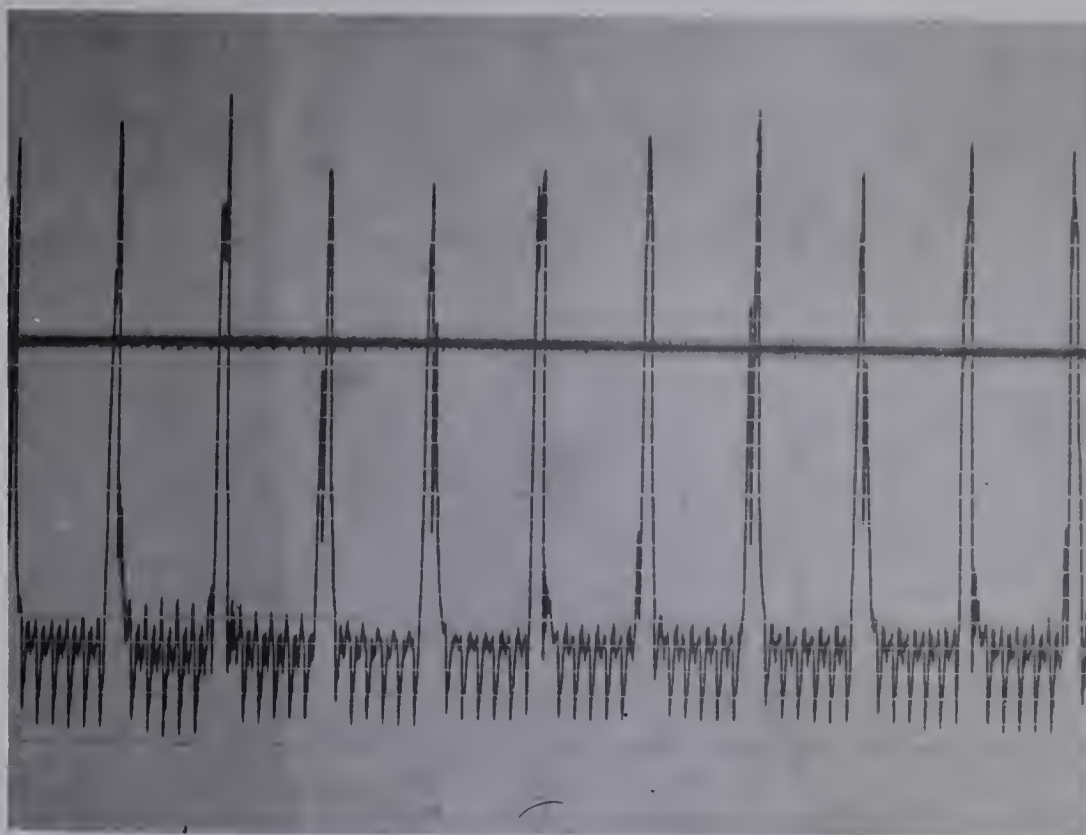


Figure 47. Isolated Lung Experiment A. Perfusion pressure tracing. High peaks coincide with inflation.

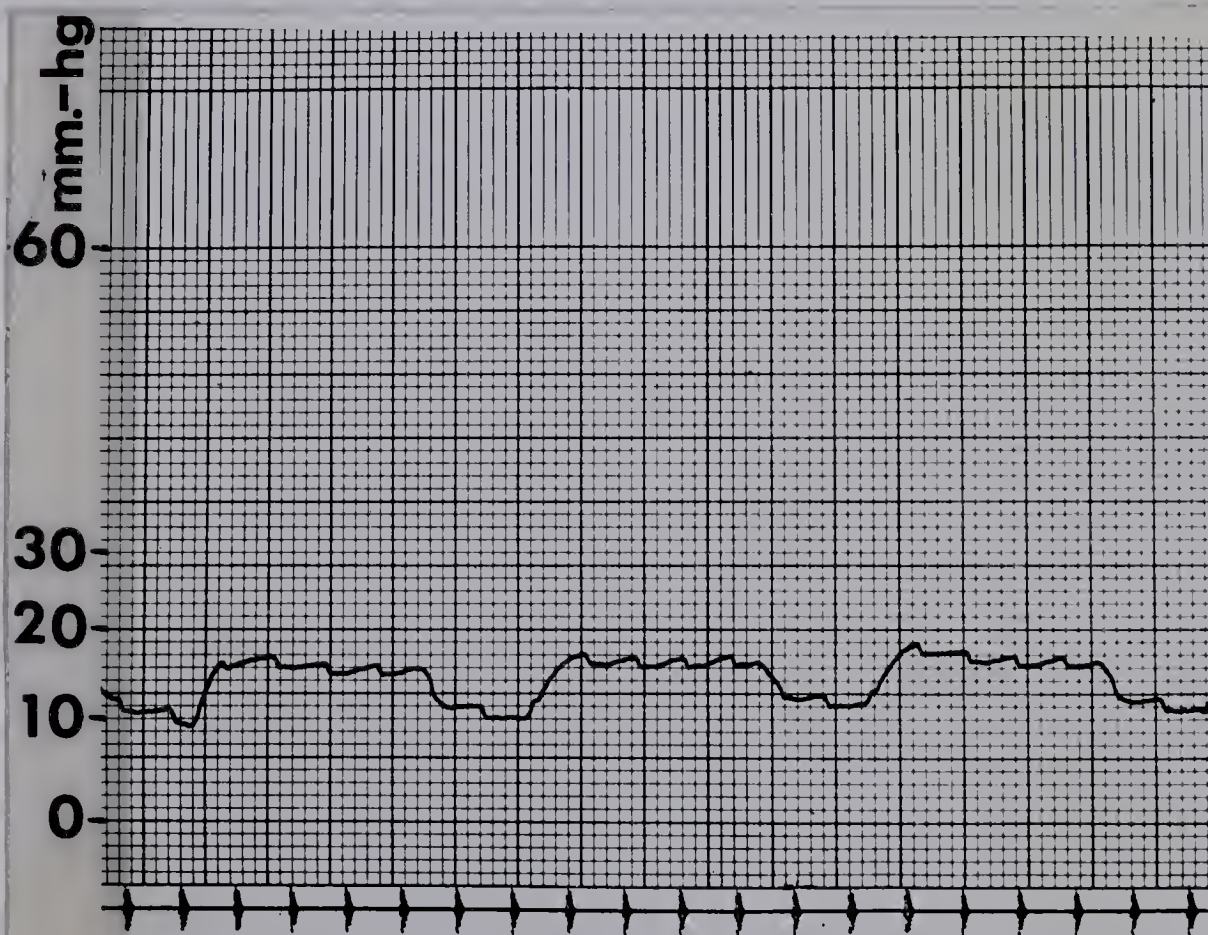


Figure 48. Isolated Lung Experiment B. Perfusion pressure inflation occurred at (a), (b) and (c).





49 and 50. Specific observations are tabulated in Addendum figures 1 to 4 and Addendum table 6.

Plasma histamine prior to the start of perfusion was within normal limits for the dog (10-20  $\mu\text{g}\%$ ). From a mean of 12.05  $\mu\text{g}\%$ , plasma histamine rose within 15 minutes to a mean of 14.33 ( $F > = 0.05$  to 0.025) remaining relatively constant thereafter, until 180 minutes when it again rose to 15.36  $\mu\text{g}\%$ .

Pre-perfusion  $\text{Na}^+$  level was 146.4 mEq/L, well within 95% of the normal dog range.  $\text{Na}^+$  remained constant, with small variations until 120 minutes when it rose slightly to 150.1 mEq/L, falling again at 180 minutes to 148.9 mEq/L.

Plasma  $\text{K}^+$  rose precipitously from 4.48 mEq/L pre-perfusion (95% of normal range = 2.6 to 4.7 mEq/L) to 4.95 mEq/L during the first 15 minutes of perfusion. By 180 minutes the mean  $\text{K}^+$  was 5.92 mEq/L. ( $F >$  for 0 minutes to 15 minutes change = 0.05 to 0.025; for 0 minutes to 180 minutes, 0.025 to 0.005).

Because of the difficulty in maintaining circulating volume in the apparatus,  $\text{Cl}^-$  determinations were performed in only a few experiments. Results are tabulated in Addendum table 6.

#### Tissue biochemical studies.

Lung tissue histamine studies confirmed that histamine appearing in the perfusate was released from the perfused organ (Table 11). The mean control tissue histamine was 44.94  $\mu\text{g}\%$ , while post-perfusion mean tissue histamine was 24.60  $\mu\text{g}\%$ .

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\* see Addendum page



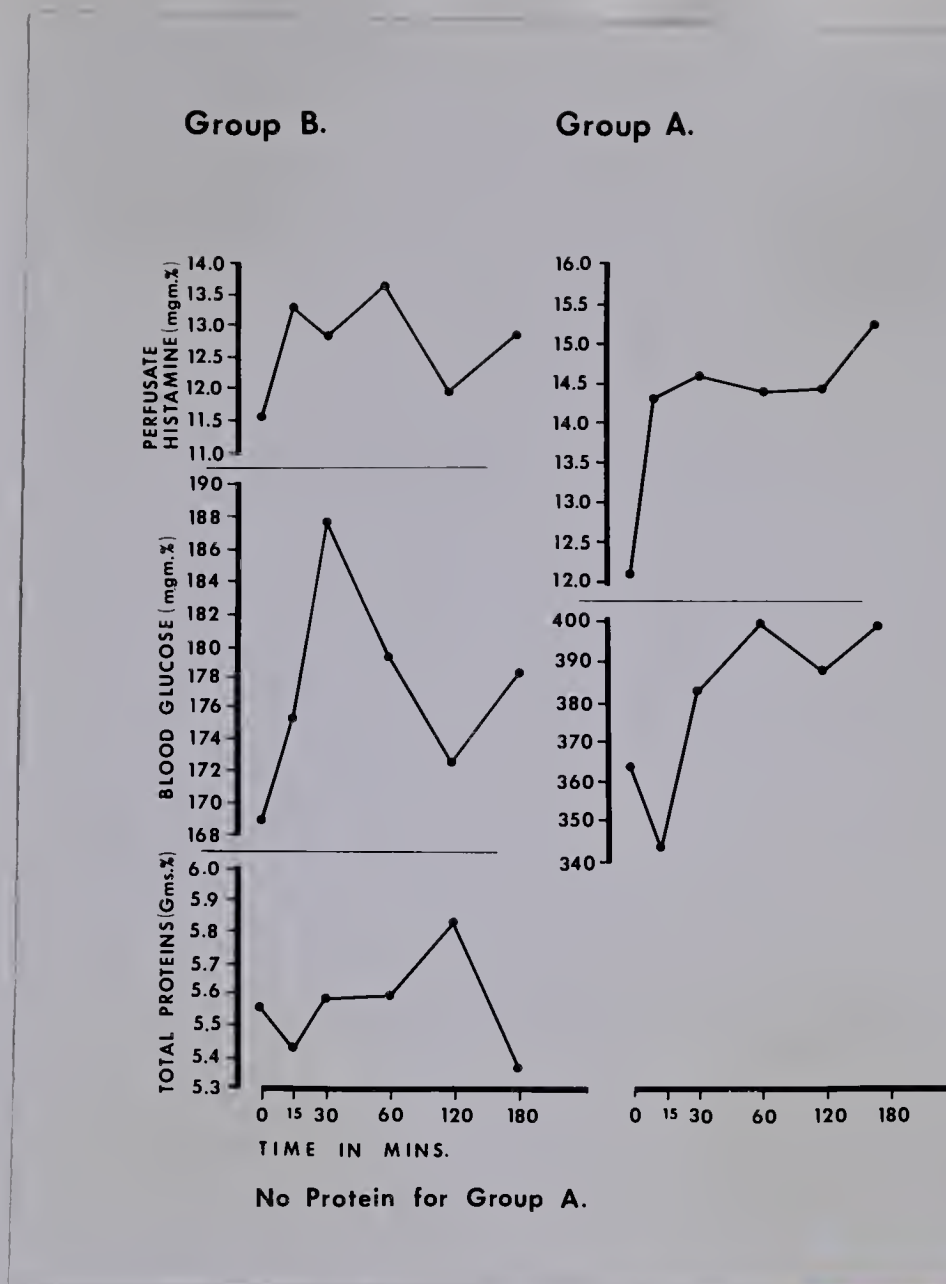


Figure 49. Isolated Lung Experiments.  
Summary of perfusate bio-  
chemical results.





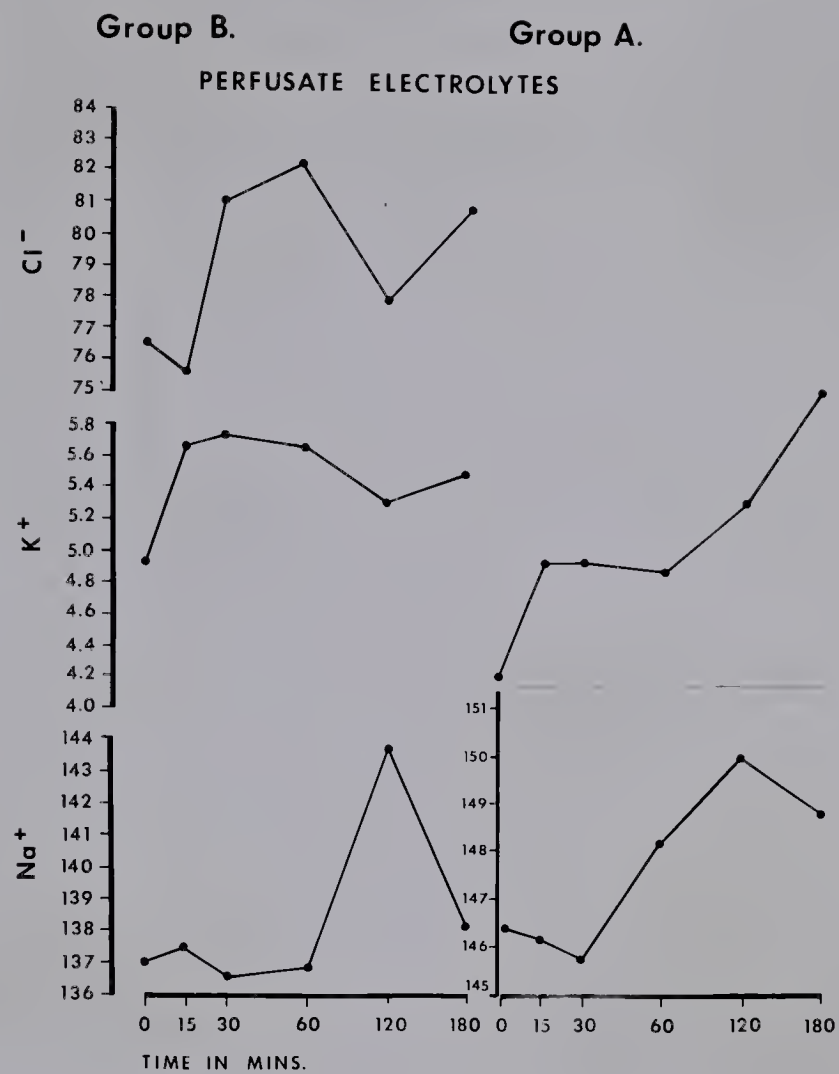


Figure 50. Isolated Lung Experiments.  
Summary of biochemical re-  
sults.



TABLE 11

TISSUE HISTAMINE DETERMINATIONS

Group	Exp. No.	Tissue Histamine ( $\mu$ g./gm.ADT)
Control	1	69.9
	2	62.1
	3	53.17
	4	43.20
	5	60.40
	6	54.50
	7	27.23
	<u>7</u>	<u>27.23</u>
	x	44.94
Isolated Lung Experiment A.	1	12.74
	2	30.17
	3	18.75
	4	54.47
	5	21.20
	6	13.70
	7	17.50
	8	54.47
	9	12.66
	10	29.24
	11	16.94
	12	13.69
	13	13.77
	14	24.46
	15	35.17
	<u>15</u>	<u>35.17</u>
	x	24.60
Isolated Lung Experiment B.	1	10.92
	2	10.82
	3	15.90
	4	26.02
	5	23.45
	6	20.31
	7	18.81
	8	25.92
	9	44.83
	10	14.32
	<u>10</u>	<u>14.32</u>
	x	20.33



Tissue  $\text{Na}^+$  rose post-perfusion to 0.492 mEq/gm. acetone dried tissue (ADT) from 0.310 mEq/gm. ADT ( $F \geq 0.10$  to 0.05), a result difficult to explain, since perfusate  $\text{Na}^+$  was also slightly higher at the end of perfusion than before perfusion. Tissue  $\text{K}^+$  fell post-perfusion from 0.189 to 0.166 mEq/gm. ADT, ( $F \geq 0.10$  to 0.05) in keeping with the observed perfusate  $\text{K}^+$  rise. Tissue  $\text{Cl}^-$  rose from 0.188 to 0.229 mEq/gm. ADT post-perfusion (Tables 12,13). Tissue hexosamine fell from a mean of 673.63 to 478.10 mgm./100 gm. dry tissue weight, while tissue hydroxyproline rose slightly from 1283.96 to 1379.99 mgm./100gm. dry tissue weight (Table 14).

#### ISOLATED LUNG EXPERIMENT B.

Again, under somewhat different experimental conditions than those prior in the above experiment, (autologous blood perfusate instead of homologous plasma, negative instead of positive pressure ventilation) pulmonary edema and approximate doubling of lung weight after perfusion was observed (Table 10). As assessed by direct observation, however, edema occurred later in perfusion (at about 40 minutes).

#### Blood gas studies. (Tables 15,16,17).

Blood pH and  $\text{PCO}_2$  followed essentially the same pattern as that observed in isolated lung experiment A., i.e., as perfusion time progressed  $\text{PCO}_2$  rose slightly and pH fell slightly.  $\text{PO}_2$  was considerably higher than in Experiment A., presumably due to the presence of red blood cells in the perfusate.





TABLE 12

TISSUE ELECTROLYTES - CONTROL

(mEq/Gm. ADT)

Exp. No.	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>
1	.300	.180	.178
2	.307	.191	-
3	.331	.174	-
4	.279	.148	.151
5	.338	.198	.244
6	.293	.123	.140
7	.307	.118	.135
8	.336	.208	.200
9	.317	.202	.203
10	.375	.232	.228
11	.296	.249	.193
<u>12</u>	<u>.336</u>	<u>.249</u>	<u>.207</u>
x	.310	.189	.188

Control - Exsanguinated

1	.226	.300	-
2	.222	.480	-
3	.427	.132	.292
4	.277	.196	.187
5	.283	.199	.194
<u>6</u>	<u>.374</u>	<u>.249</u>	<u>.263</u>
x	.301	.259	.234



TABLE 13

TISSUE ELECTROLYTES

(mEq/Gm. ADT)

Group	Exp. No.	Na <sup>+</sup>	K <sup>+</sup>	Cl <sup>-</sup>
Isolated Lung Experiment A.	1	.504	.0415	.306
	2	.526	.194	.296
	3	.460	.203	.266
	4	.472	.155	.135
	5	.478	.143	.150
	6	.316	.312	.165
	7	.627	.271	.081
	<u>8</u>	<u>.554</u>	<u>.244</u>	<u>.181</u>
	x	.492	.229	.166
Isolated Lung Experiment B.	1	.390	-	.286
	2	.606	.246	.228
	3	.414	.170	.101
	4	.416	.170	.160
	5	.460	.106	.104
	6	.379	.156	.436
	7	-	-	.755
	8	-	-	.289
	9	.303	.147	.152
	<u>10</u>	<u>.385</u>	<u>.161</u>	<u>.221</u>
	x	.419	.165	.273





TABLE 14

CONNECTIVE TISSUE STUDIES

Group	Exp. No.	Hydroxy- proline (mgm/100gm dry weight)	Hexosamine (mgm/100gm dry weight)
Control	1	967.582	33.466
	2	817.863	663.472
	3	2086.92	845.969
	4	1797.73	558.68
	5	1942.45	755.58
	6	1180.37	893.67
	7	1360.40	691.39
	8	1532.98	739.53
	9	1136.28	1014.53
	<u>10</u>	<u>689.18</u>	<u>763.82</u>
	x	1283.96	673.63
Isolated Lung Experiment A.	1	1187.47	91.848
	2	1489.94	640.41
	3	1058.85	670.04
	4	1434.33	468.01
	5	1358.00	497.33
	6	1121.49	514.94
	<u>7</u>	<u>2009.83</u>	<u>464.11</u>
	x	1379.99	478.10
Isolated Lung Experiment B.	1	1436.63	711.50
	2	1566.29	689.69
	3	1836.40	704.58
	4	2717.26	729.20
	<u>5</u>	<u>1289.40</u>	<u>261.36</u>
	x	1769.19	619.26



TABLE 15

ISOLATED LUNG EXPERIMENT B - BLOOD pH

Exp. No.	Time (Minutes)					
	0	15	30	60	120	180
1	7.27	7.15	7.13	7.18	7.14	7.14
2	7.43	7.13	7.06	7.06	7.07	7.07
3	7.49	7.21	7.17	7.16	7.17	7.16
4	7.43	7.20	7.15	7.14	7.15	7.13
5	7.48	7.15	7.11	7.08	7.07	7.07
6	7.64	7.27	7.18	7.20	7.21	7.27
7	7.55	7.32	7.20	7.16	7.15	7.15
8	7.36	7.26	7.19	7.17	7.17	7.17
9	7.41	7.24	7.22	7.17	7.15	7.15
<u>10</u>	<u>7.45</u>	<u>7.23</u>	<u>7.15</u>	<u>7.12</u>	<u>7.13</u>	<u>7.12</u>
x 37°C	7.451	7.216	7.156	7.144	7.141	7.143
x 15°C		7.539	7.479	7.467	7.464	7.466



TABLE 16

ISOLATED LUNG EXPERIMENT B - BLOOD  $P_{CO_2}$

Exp. No.	Time (Minutes)					
	0	15	30	60	120	180
1	44.2	63.9	65.3	55.6	58.8	62.2
2	34.9	61.7	68.2	68.2	68.6	64.2
3	25.8	58.2	61.1	64.3	60.4	58.9
4	30.1	56.3	64.2	66.8	64.3	67.9
5	17.4	58.2	62.6	69.2	70.8	66.4
6	14.9	43.5	54.5	50.0	47.9	39.1
7	25.2	36.8	53.2	59.4	61.1	61.8
8	37.8	44.9	57.5	61.4	58.9	56.1
9	31.3	48.3	47.9	57.5	65.6	65.9
<u>10</u>	<u>28.3</u>	<u>53.2</u>	<u>65.8</u>	<u>64.9</u>	<u>63.7</u>	<u>67.6</u>
x $37^{\circ}C$	28.89	52.50	60.03	61.73	62.01	61.01
x $15^{\circ}C$	-	33.08	37.82	38.89	39.07	38.44





TABLE 17

ISOLATED LUNG EXPERIMENT B - BLOOD  $P_{O_2}$

Exp. No.	Time (Minutes)					
	0	15	30	60	120	180
1	-	752	755	742	769	743
2	66	798	748	875	843	806
3	92	779	799	812	828	819
4	87	795	827	806	773	863
5	288	746	781	778	791	839
6	101	670	703	709	706	631
7	94	731	820	826	858	834
8	68	754	723	683	742	730
9	69	806	845	873	871	872
<u>10</u>	<u>75</u>	<u>738</u>	<u>828</u>	<u>793</u>	<u>648</u>	<u>831</u>
x 37 <sup>O</sup> C	104.4	756.9	782.9	789.7	782.9	796.8



### Pressure-flow relationships

Under constant flow conditions, no initial rise in perfusion pressure was noted when autologous blood perfusate was used. Pulmonary artery pressure fell rapidly during the first 15 minutes and then more gradually over the next 1 3/4 hours from a mean of 17.52 to a mean of 12.37 and then rose to a mean of 14.51 by three hours (Fig. 51). Again the marked influence of inflation pressure on perfusion pressure was noted (Fig. 48), except that with negative pressure inflation perfusion pressure fell with inflation.

The same pattern of perfusate biochemical changes was seen in experiment B as in experiment A (Figs 49,50). Perfusate histamine rose rapidly from a mean of 11.56  $\mu\text{g}\%$  to 13.33  $\mu\text{g}\%$  within 15 minutes of commencement of perfusion, fell slightly to 12.82 at 30 minutes, rose to 13.70 at 60 minutes ( $F > 0.05$  to  $-0.025$  for 0 minutes to 60 minutes change), fell to 11.92 at 120 minutes, and rose to 12.84  $\mu\text{g}\%$  at 180 minutes. Tissue histamine was markedly diminished post-perfusion (Table 12). If mean value curves for perfusate glucose,  $\text{K}^+$  and  $\text{Cl}^-$  are compared with the perfusate histamine curve (Fig. 49), it is immediately apparent that as perfusate histamine rises, glucose,  $\text{K}^+$  and  $\text{Cl}^-$  also rise significantly ( $F >$  for glucose 0 to 30 minute change 0.05 to 0.025, for  $\text{K}^+$ , 0.01 to 0.005). Furthermore, as fluctuations in perfusate histamine occur, glucose,  $\text{K}^+$  and  $\text{Cl}^-$  change in the same direction. Total protein and  $\text{Na}^+$  curves are approximate mirror images of the perfusate histamine curve. Tissue electrolyte results are essentially the same





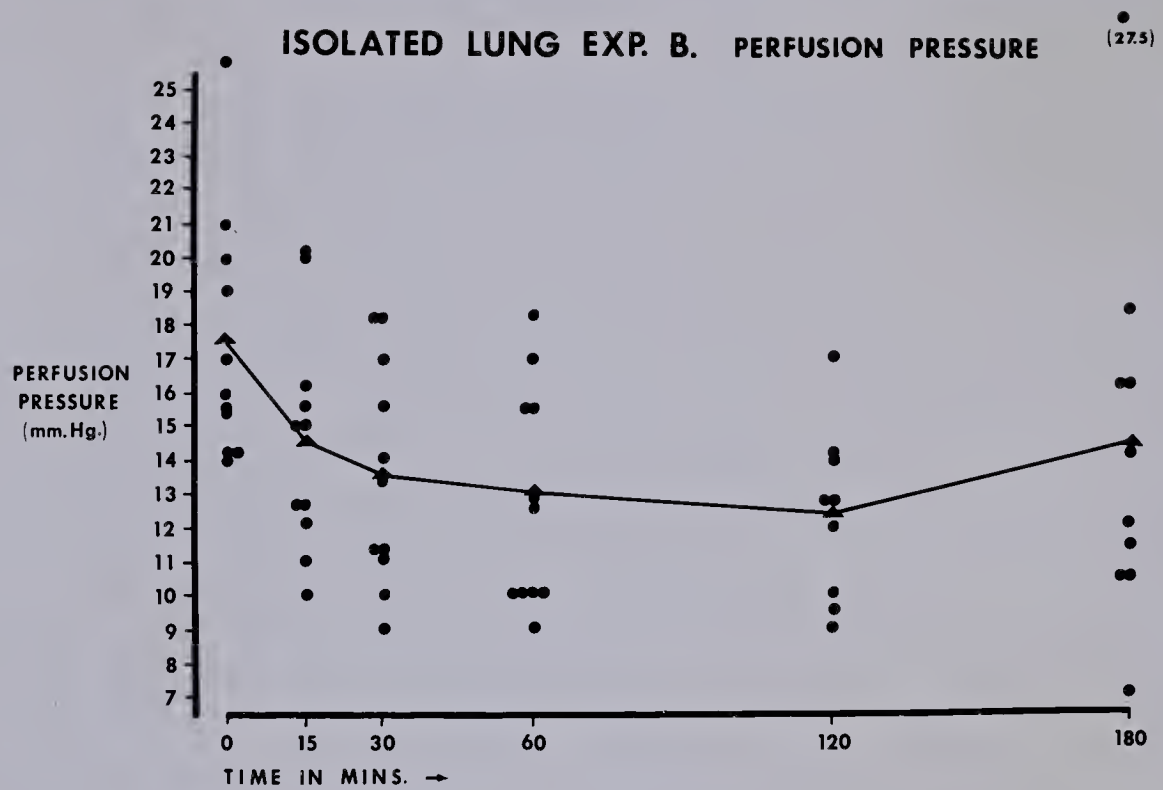


Figure 51. Isolated Lung Experiment - Perfusion Pressure.



as those obtained in experiment A., i.e., Mean  $K^+$  0.165,  $Na^+$  0.419 and  $Cl^-$  0.273 mEq/gm. ADT (Table 14).

Since no external source of the perfusate constituents measured existed, changes in perfusate levels of these substances must be governed by events occurring in the hypothermic perfused and ventilated lung. That histamine, potassium and reducing substances are released by the lung under the imposed conditions is definite. Reducing substances, other than glucose, may be measured by the Folin-Wu method utilized in these experiments. However, this "saccharoid" blood fraction is almost entirely accounted for by glutathione and glucuronic acid. It seems most unlikely that these substances could account for the observed perfusate glucose changes.

A number of possibilities present themselves as explanations for the mechanism of histamine release from the lung. Histamine is normally found in all mammalian tissues in a bound and inactive form (233). Lung is second only to the central nervous system in tissue histamine content (234). In intact dogs transfused with homologous plasma, Bliss, Johns and Carmen showed histamine release due to an individual-specific plasma factor, analogous to an immediate hypersensitivity reaction. Histamine release under similar conditions has also been demonstrated in humans (235). This reaction was thought initially to be the cause of the histamine release seen in isolated lung experiment A. However, since release also occurred when autologous blood was used as a perfusate,





other factors must play a role. Tissue anoxia may have occurred since at 15°C oxygen availability is markedly diminished, and where plasma perfusate was used, although plasma  $P_{O_2}$  was high, plasma  $O_2$  content was probably low. Respiratory alkalosis may also have been a stimulus for histamine release. Also, since a pure  $O_2$ - $CO_2$  mixture was used to ventilate all lungs, little  $N_2$  existed in the system. Dog basophils contain 1080  $\mu g$  of histamine per  $10^9$  cells (236). Trauma to these cells may have produced the observed effects. Donor animals for both plasma and blood were anesthetized with pentothal. Ruch and Fulton suggest barbiturates as a cause of histamine release from tissues (236) as well as tissue mutilation as is seen in trauma (233). It may also be that the dog lung normally releases histamine, and that the observed effects are not due to organ trauma at all. The perfusion pressure curve was abnormal. The marked influence of inflation pressure and method on perfusion pressure can be explained by the "sluice hypothesis" (195). When high positive inflation pressures are used, venous pulmonary outflow can be temporarily occluded, explaining the marked rise in pulmonary artery pressure during inflation in Group A. experiments. Similarly, negative pressure inflation produces a fall in pulmonary vascular resistance if inflation pressure is not greatly negative (237), explaining the fall in perfusion pressure with inflation seen in Group B. experiments. This abnormal hemodynamic effect of isolated lung ventilation might provide a stimulus for





histamine release and be important in the genesis of isolated lung edema.

The observed changes in connective tissue components also suggest that tissue permeability changes play a major role in perfused organ edema. Hydroxyproline is generally regarded as an index of tissue collagen content. Similarly, hexosamine is regarded as a good indication of tissue mucopolysaccharide content, i.e., "ground substance" (248).

Considerable evidence supports the suggestion that the physico-chemical state of the non-cellular ground substance determines to a great extent tissue permeability. Chiricuta et al., were able to correlate observed changes in the mucopolysaccharides of capillary walls of various organs with the onset of edema following experimental burns (250). Douglas et al., observed similar changes in hydroxyproline and hexosamine (i.e. an increase in hydroxyproline and a fall in hexosamine) in the walls of arteries following depletion of mast-cells (and hence histamine) from animals treated with 48/80 (249).

Several explanations are possible for the unexpected fall in perfusion pressure seen in both Group A and B experiments as perfusion time progressed. Acute hypoxia increases pulmonary vascular resistance in both intact animals and in isolated lungs (194). The anoxia produced while removing the lung from the animal and transferring it to the perfusion apparatus may have produced increased organ vascular pressure

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initially, which was relieved as perfusion progressed. Woodbury and Hamilton (238), under conditions of low pulmonary blood flow, (as in our experiments) observed a transient rise, then a prolonged fall in pulmonary artery pressure following injection of histamine. "The pulmonary artery pressure pulses became mere ripples and the pulmonary pressures were reduced", effects also seen in the current experiments (Fig. 48).

Normally, the cell membrane does not allow an easy passage of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cl}^-$  and glucose in and out of the cell. Therefore one must postulate some mechanism to account for the rapid shift in perfusate-tissue electrolytes and glucose and/or reducing substances. The cell membrane "sodium-potassium" pump maintains intracellular potassium high and sodium low through the utilization of metabolic energy obtained from oxidation-reduction reactions (239). Within the cell glucose undergoes an obligatory irreversible conversion to glucose-6-phosphate (240) which under normal circumstances, cannot be released from lung tissue (241).

Hypothermia affects intracellular electrolyte balance since respiration is inhibited with reduction in temperature, and it is respiration which furnishes the energy necessary for the exclusion of certain ions and uptake of others by the cell membrane. Anesthetics may produce the same respiratory alterations (242).

Histamine is termed a "permeability factor" along with hydroxytryptamine, proteases, and certain products of proteolysis. Considerable overlap exists since polypeptides may act







as histamine releasors (243). The increased capillary permeability produced by histamine has been attributed to capillary dilatation, although this idea is not accepted by all investigators (244). The injury produced leads to increased capillary permeability, pressure and flow, with eventual stasis, capillary blockage and passive congestion. Recovery from stasis cannot occur until capillary wall permeability to protein is lowered toward normal (245). During isolated lung perfusion sufficient histamine was released to produce the above hemodynamic changes. In addition, since the lung is incapable of inactivating histamine (246), it continues to circulate and exert its action with each passage through the lung.

That perfusate protein falls as histamine rises could mean loss of protein into the extravascular compartment due to vasodilatation. As the upset of tissue electrolytes and other constituents proceeds, a vicious circle of events could be initiated in which metabolic alterations cause more histamine release, which in turn produces more metabolic alterations.

Since the lungs are deprived of their pulmonary circulation during cardio-pulmonary bypass, histamine released by the lungs during the procedure would remain in the pulmonary vasculature in an active form, producing some or all of the changes observed in isolated lung experiments. The pulmonary edema and other pulmonary complications seen following cardiac surgery might be explained on this basis. In fact, histamine and 5-hydroxytryptamine have already been implicated by indirect



means in the etiology of these complications (247).

Since cell membrane permeability changes are probably among the earliest to develop as cells die, they may also represent a very early index of transplant tissue viability.

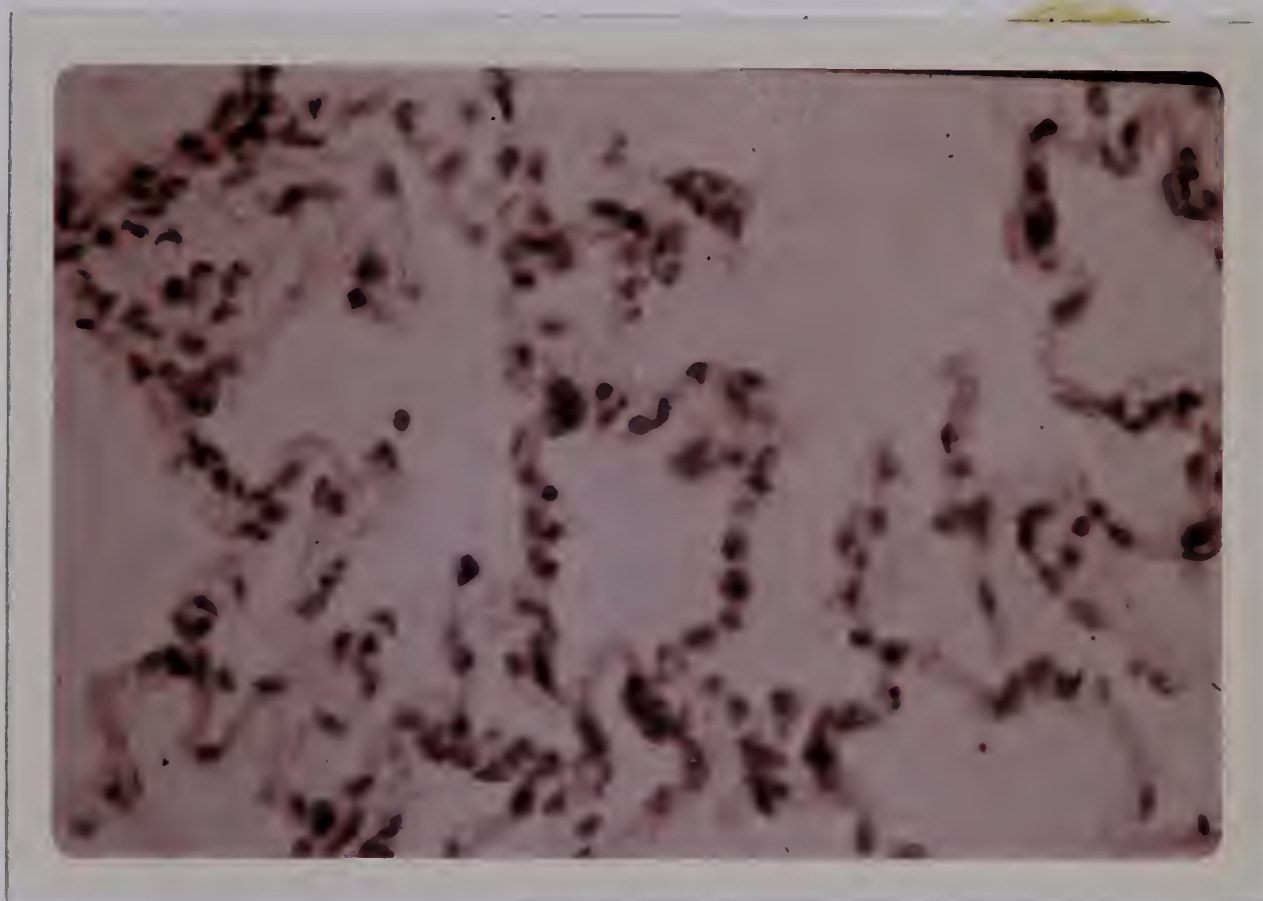


Figure 52. Isolated Lung Experiment A. Perfused ventilated and hypothermic lung histology (high power). Despite the presence of gross post-perfusion edema, accompanied by marked tissue metabolic alterations, alveolar architecture is well preserved. No atelectasis or congestion was noted in any section examined.





## CHAPTER IV

### SUMMARY AND CONCLUSIONS



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Under the imposed experimental conditions left lung autotransplantation produces no impairment in pulmonary function save the loss of the left Hering-Breuer reflex. Arterial blood pH,  $\text{PaCO}_2$ , and  $\text{HbO}_2$  saturation values do not differ significantly from those obtained in control animals. No post-operative increase in  $(a-A)\text{CO}_2$  or  $(A-a)\text{O}_2$  was demonstrated. Grossly normal graft perfusion was demonstrated by pulmonary angiography and no significant elevation in pulmonary artery pressure was seen following operation. Graft ventilation and oxygen uptake are not impaired in the late post-operative period. Barbiturate anaesthesia markedly disturbs  $\dot{V}_A/\dot{Q}$  (pulmonary ventilation-perfusion relationships) and probably increases  $(A-a)\text{O}_2$  markedly, both in control and operated animals.

Intravascular perfusion produces rapid graft hypothermia, theoretically reducing graft metabolism to approximately 25% of normal within five to ten minutes.

Under the imposed conditions left lung autografts will regularly survive four hours and occasionally six hours of hypothermic preservation. Preservation for longer than six hours under these conditions has not been successful. Factors involved in graft failure may include graft death prior to reimplantation, vascular thrombosis and hemorrhagic pulmonary infarction and loss of graft alveolar surfactant.

Although the series was small, the addition of hyperbaric oxygenation to graft hypothermia did not appear to alter



graft survival. Graft vessel thrombosis appeared to be probably the chief cause of mortality in this group.

When the preserved autograft survives, its function is indistinguishable from that of the immediately autotransplanted lung, as assessed by arterial pH,  $p\text{CO}_2$ ,  $p\text{O}_2$ ,  $\text{HbO}_2$ , saturation, pulmonary angiography,  $(a-A)\text{CO}_2$ ,  $(A-a)\text{O}_2$ , differential broncho-spirometry and right heart catheterization. That is, transplant perfusion ventilation and gas exchange appear to take place and are within normal limits, despite the loss of the Hering-Breuer reflex on the operated side.

Using the described method of simultaneous lung perfusion, ventilation and profound hypothermia, successful lung preservation for extended periods could not be achieved due to graft weight gain and edema. Under the imposed conditions, graft temperature can be accurately regulated.

Acid-base balance in the isolated lung system can be maintained by the addition of  $\text{CO}_2$  to the isolated lung inspired air when either plasma or blood are used as perfusates.

Air embolization produces a rapid and sustained elevation of perfusion pressure and the rapid onset of edema in the perfused lung.

Unconjugated histamine is released by the lung into the perfusate. As isolated perfusion time progresses, perfusion pressure declines, probably as an effect of histamine release or pulmonary anoxia prior to perfusion. Potassium and reducing substances, probably glucose, are also released from the perfused







lung. Post-perfusion tissue sodium is markedly elevated.

Following hypothermic perfusion and ventilation, pulmonary tissue hydroxyproline rose and hexosamine fell, correlating with the observed histamine release and tissue permeability alterations.

The hemodynamics of the isolated perfused hypothermic lung are markedly abnormal, and the vascular effects of ventilation are pronounced whether inflation is by positive or negative pressure.

Perfusate gas studies indicate adequate perfusate oxygenation, but as pulmonary edema progresses, gas exchange is impaired.

On the basis of these studies the observed biochemical alterations and isolated lung edema can be explained at least in part by the effects of profound hypothermia, histamine release and abnormal hemodynamics of the perfused and ventilated hypothermic lung.



## CHAPTER V

## ADDENDUM

1992  
1993

ADDENDUM TABLE 1

Isolated Lung Experiment A

PERFUSION PRESSURE (mm/Hg)

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
1	24.3	21.0	20.0	16.0	13.0	-
2	20.5	19.5	20.0	17.2	16.0	-
3	22.0	20.0	20.0	20.0	20.0	21.0
4	20.5	27.5	27.5	27.5	27.5	26.0
5	22.0	27.0	30.0	26.0	24.0	24.5
6	17.0	-	12.0	11.5	11.0	11.0
7	21.5	20.0	17.0	-	-	-
8	11.5	9.0	8.5	-	-	-
9	17.0	11.0	11.0	10.0	8.5	-
10	<u>21.0</u>	<u>20.0</u>	<u>19.5</u>	<u>18.5</u>	<u>17.0</u>	<u>-</u>
$\bar{x}$	19.73	19.44	18.55	18.33	17.12	18.25



TABLE 1  
Isolated Long Corals  
(Long Corals, Table 1)

Isolated Long Corals						Depth (m)
1961	1962	1963	1964	1965	1966	
-	0.01	0.01	0.01	0.13	1.04	1
-	0.01	0.01	0.01	0.01	0.01	2
0.13	0.01	0.01	0.01	0.01	0.01	3
0.01	0.01	0.01	0.01	0.01	0.01	4
0.01	0.01	0.01	0.01	0.01	0.01	5
0.01	0.01	0.01	0.01	-	0.01	6
-	-	-	0.01	0.01	0.01	7
-	-	-	0.01	0.01	0.01	8
-	0.01	0.01	0.01	0.01	0.01	9
-	0.01	0.01	0.01	0.01	0.01	10
0.01	0.01	0.01	0.01	0.01	0.01	11

ADDENDUM TABLE 2

Isolated Lung Experiment A  
PRESSURE FLOW RELATIONSHIP  
(mm/Hg/cc per minute)

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
1	.108	.093	.057	.046	.037	.031
2	.205	.195	.200	.172	.160	.160
3	.220	.200	.200	.200	.200	.210
4	.205	.275	.275	.275	.275	.260
5	.220	.270	.300	.260	.240	.245
6	.170	-	.120	.115	.110	.110
7	.215	.200	.170	-	-	-
8	.115	.090	.085	-	-	-
9	.043	.028	.028	.025	.040	-
10	<u>.210</u>	<u>.200</u>	<u>.195</u>	<u>.185</u>	<u>.170</u>	<u>-</u>
$\bar{x}$	.171	.1723	.1630	.1597	.1540	.1693

TABLE 1

TABLE 1  
 SUMMARY OF DATA  
 (continued)

Time (minutes)						Run
0	10	20	30	40	50	
100	100	100	100	100	100	1
100	100	100	100	100	100	2
100	100	100	100	100	100	3
100	100	100	100	100	100	4
100	100	100	100	100	100	5
100	100	100	100	100	100	6
100	100	100	100	100	100	7
100	100	100	100	100	100	8
100	100	100	100	100	100	9
100	100	100	100	100	100	10
100	100	100	100	100	100	11
100	100	100	100	100	100	12
100	100	100	100	100	100	13
100	100	100	100	100	100	14
100	100	100	100	100	100	15
100	100	100	100	100	100	16
100	100	100	100	100	100	17
100	100	100	100	100	100	18
100	100	100	100	100	100	19
100	100	100	100	100	100	20

ADDENDUM TABLE 3

Isolated Lung Experiment A

PLASMA  $P_{CO_2}$

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
	12.25	120	-	88	98	99
1	34.6	-	42.3	42.6	-	-
2	39.1*	37.7	37.3	40.2	-	-
3	-	34.1	33.9	38.7	-	-
4	32.5	38.2	45.3	41.4	42.5	-
5	30.9	35.7	34.7	-	-	-
6	24.4	38.4	35.0	41.3	-	-
7	<u>16.1</u>	<u>24.7</u>	<u>25.3</u>	<u>25.3</u>	<u>-</u>	<u>-</u>
$\bar{x}_{37}^{\circ}C$	27.20	34.80	36.43	38.25		
$\bar{x}_{15}^{\circ}C$	17.14	21.93	22.95	24.10		

\* Sample taken after perfusion initiated





ADDENDUM TABLE 4

Isolated Lung Experiment A

PLASMA pH

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
1	-	7.25	7.10	7.10	7.12	7.12
2	7.65	6.95	6.95	6.95	6.98	6.99
3	7.26*	-	7.11	7.11	-	-
4	7.13	7.11	7.11	7.09	-	-
5	-	7.20	7.23	7.18	-	-
6	7.10	7.05	6.96	6.96	6.97	-
7	-	7.00	7.00	7.00	-	6.95
8	-	7.15	7.15	7.12	7.16	-
9	<u>7.41</u>	<u>7.25</u>	<u>7.27</u>	<u>7.21</u>	<u>-</u>	<u>-</u>
$\bar{x}_{37^{\circ}\text{C}}$	7.31	7.12	7.10	7.08	7.06	7.02
$\bar{x}_{15^{\circ}\text{C}}$	7.76	7.44	7.42	7.40	7.38	7.34

\* Sample taken after perfusion initiated.

# TABLE 1

Estimated Long-Term Average  
Annual Rainfall

Station	Elevation (Feet)	Annual Rainfall (Inches)				Notes
		1900	1910	1920	1930	
1	51.5	61.0	7.0	20.0	-	
2	30.0	29.0	29.0	29.0	29.0	
3	-	11.5	11.5	-	20.0	
4	-	10.0	12.5	14.5	11.5	
5	-	10.5	10.5	10.5	-	
6	10.0	10.0	10.0	10.0	10.0	
7	-	10.0	10.0	10.0	-	
8	10.0	10.0	10.0	10.0	10.0	
9	-	10.0	10.0	10.0	-	
10	10.0	10.0	10.0	10.0	10.0	
11	10.0	10.0	10.0	10.0	10.0	
12	10.0	10.0	10.0	10.0	10.0	
13	10.0	10.0	10.0	10.0	10.0	
14	10.0	10.0	10.0	10.0	10.0	
15	10.0	10.0	10.0	10.0	10.0	
16	10.0	10.0	10.0	10.0	10.0	
17	10.0	10.0	10.0	10.0	10.0	
18	10.0	10.0	10.0	10.0	10.0	
19	10.0	10.0	10.0	10.0	10.0	
20	10.0	10.0	10.0	10.0	10.0	
21	10.0	10.0	10.0	10.0	10.0	
22	10.0	10.0	10.0	10.0	10.0	
23	10.0	10.0	10.0	10.0	10.0	
24	10.0	10.0	10.0	10.0	10.0	
25	10.0	10.0	10.0	10.0	10.0	
26	10.0	10.0	10.0	10.0	10.0	
27	10.0	10.0	10.0	10.0	10.0	
28	10.0	10.0	10.0	10.0	10.0	
29	10.0	10.0	10.0	10.0	10.0	
30	10.0	10.0	10.0	10.0	10.0	
31	10.0	10.0	10.0	10.0	10.0	
32	10.0	10.0	10.0	10.0	10.0	
33	10.0	10.0	10.0	10.0	10.0	
34	10.0	10.0	10.0	10.0	10.0	
35	10.0	10.0	10.0	10.0	10.0	
36	10.0	10.0	10.0	10.0	10.0	
37	10.0	10.0	10.0	10.0	10.0	
38	10.0	10.0	10.0	10.0	10.0	
39	10.0	10.0	10.0	10.0	10.0	
40	10.0	10.0	10.0	10.0	10.0	
41	10.0	10.0	10.0	10.0	10.0	
42	10.0	10.0	10.0	10.0	10.0	
43	10.0	10.0	10.0	10.0	10.0	
44	10.0	10.0	10.0	10.0	10.0	
45	10.0	10.0	10.0	10.0	10.0	
46	10.0	10.0	10.0	10.0	10.0	
47	10.0	10.0	10.0	10.0	10.0	
48	10.0	10.0	10.0	10.0	10.0	
49	10.0	10.0	10.0	10.0	10.0	
50	10.0	10.0	10.0	10.0	10.0	

Source: United States Weather Bureau

ADDENDUM TABLE 5

Isolated Lung Experiment A

PLASMA  $P_{O_2}$

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
1	190	465	380	-	440	470
2	509*	491	566	574		
3	-	409	345	518	-	-
4	141	457	592	527	602	-
5	90	-	80	-	213	-
6	70	-	373	437	403	-
7	<u>60</u>	<u>419</u>	<u>376</u>	<u>566</u>	<u>-</u>	<u>-</u>
$\bar{x}$	110.2	448.2	401.7	524.4	414.2	470.0

\* Sample taken after perfusion initiated.



ADDENDUM TABLE 6

Isolated Lung Experiment A

PERFUSATE  $\text{Cl}^-$  (mEq/L)

Exp. No.	<u>Time (minutes)</u>					
	0	15	30	60	120	180
1	85	85	85	85	88	90
2	123	127	120	125	120	120
3	117	115	-	115	115	100

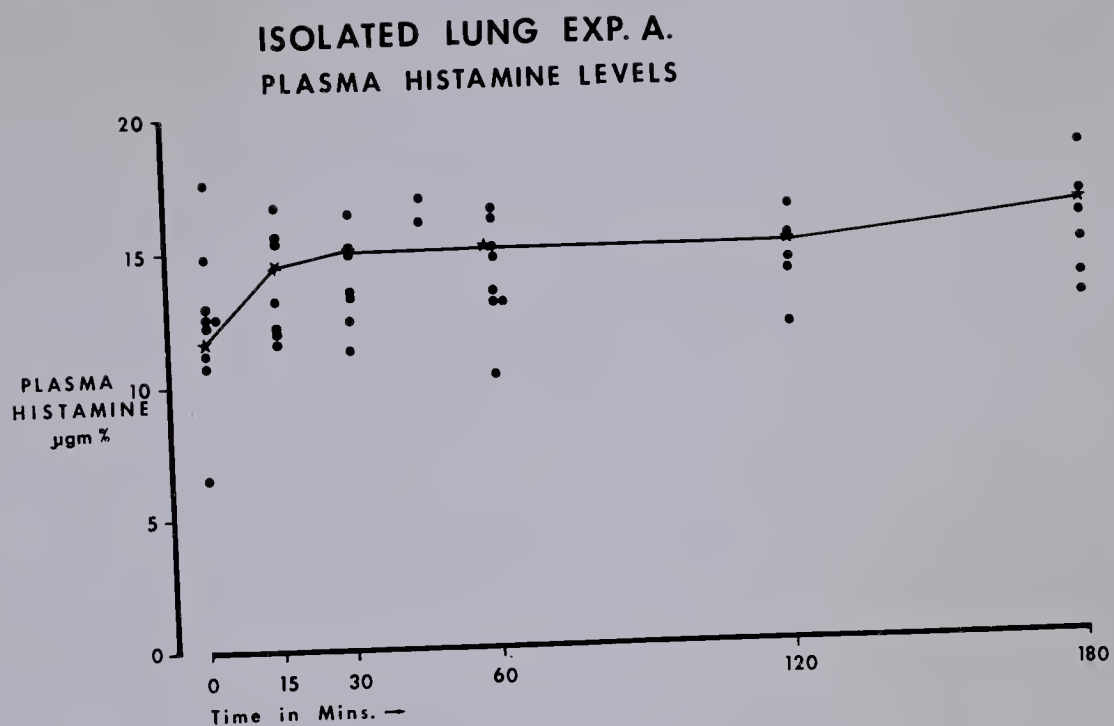


ANALYTICAL DATA

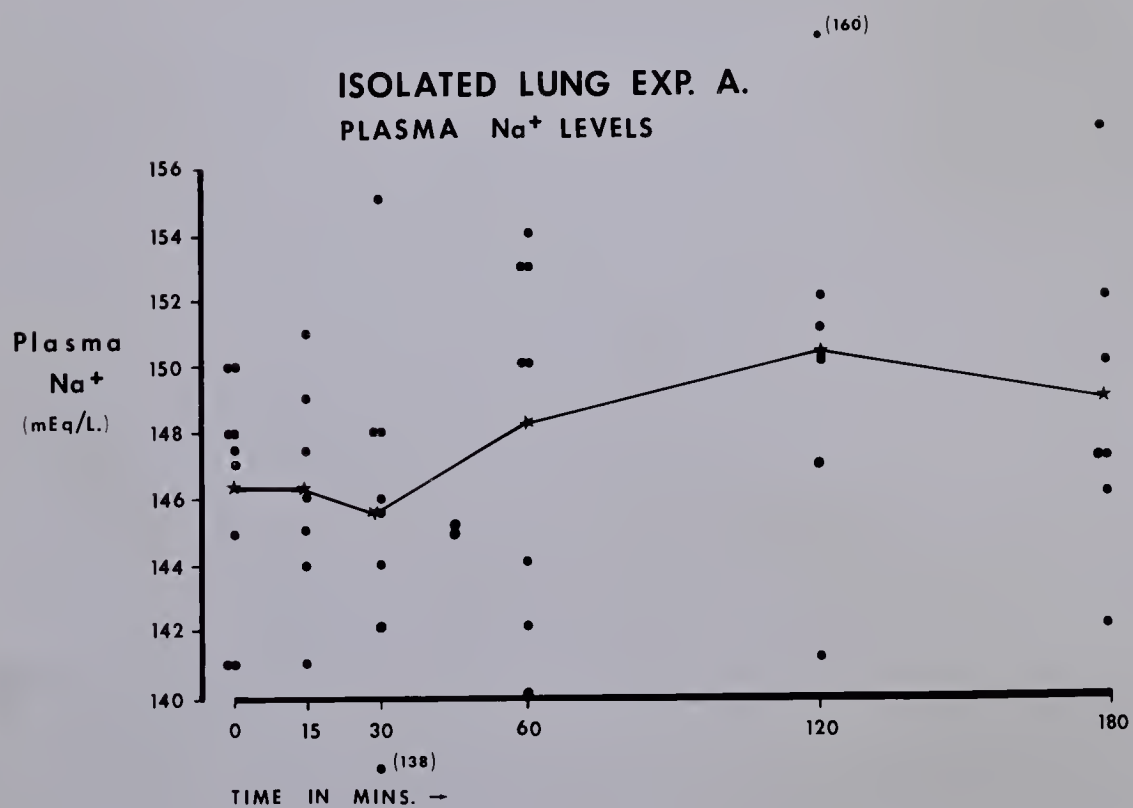
For the purpose of this study

(1) (2) (3) (4) (5) (6)

(continued)					
(1)	(2)	(3)	(4)	(5)	(6)
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100	100	100	100	100	100
100	100	100	100	100	100
100	100	100	100	100	100

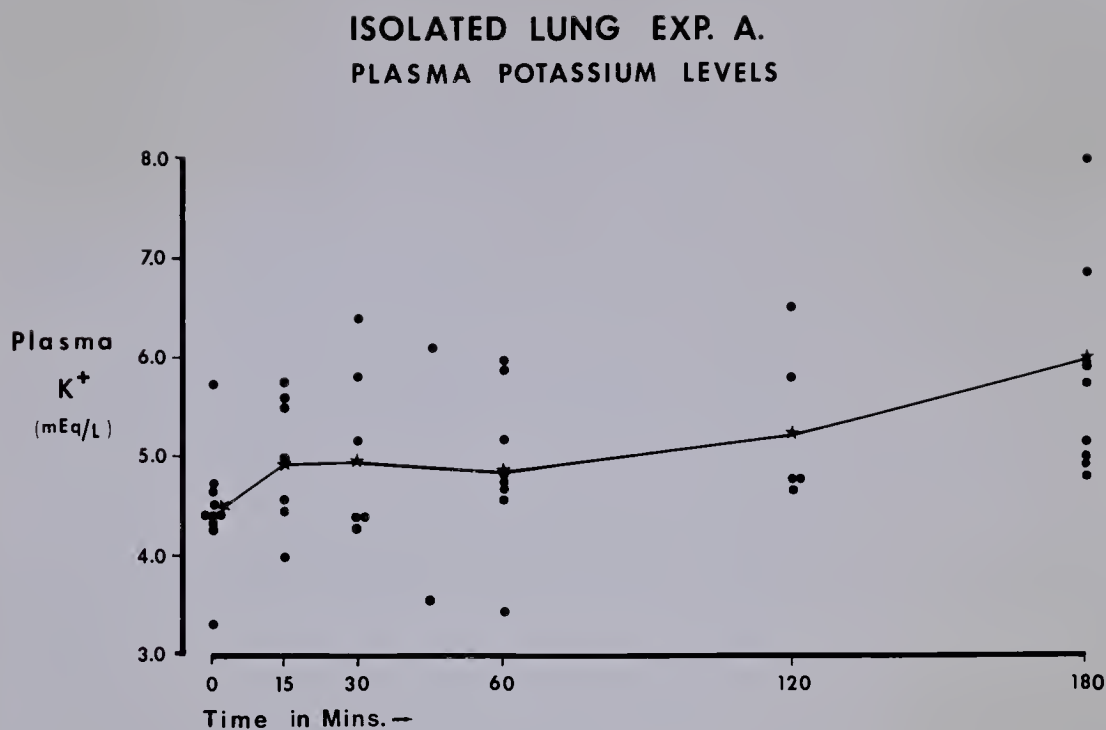


Addendum Figure 1.

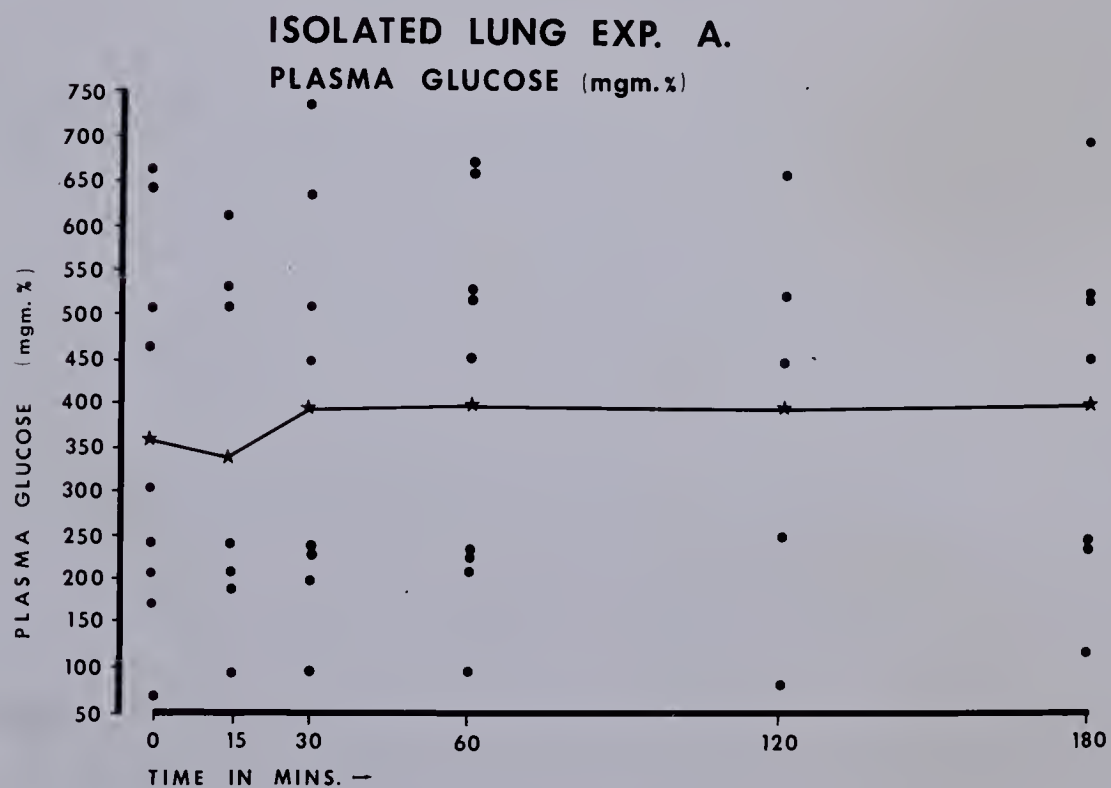


Addendum Figure 2.





Addendum Figure 3.



Addendum Figure 4.

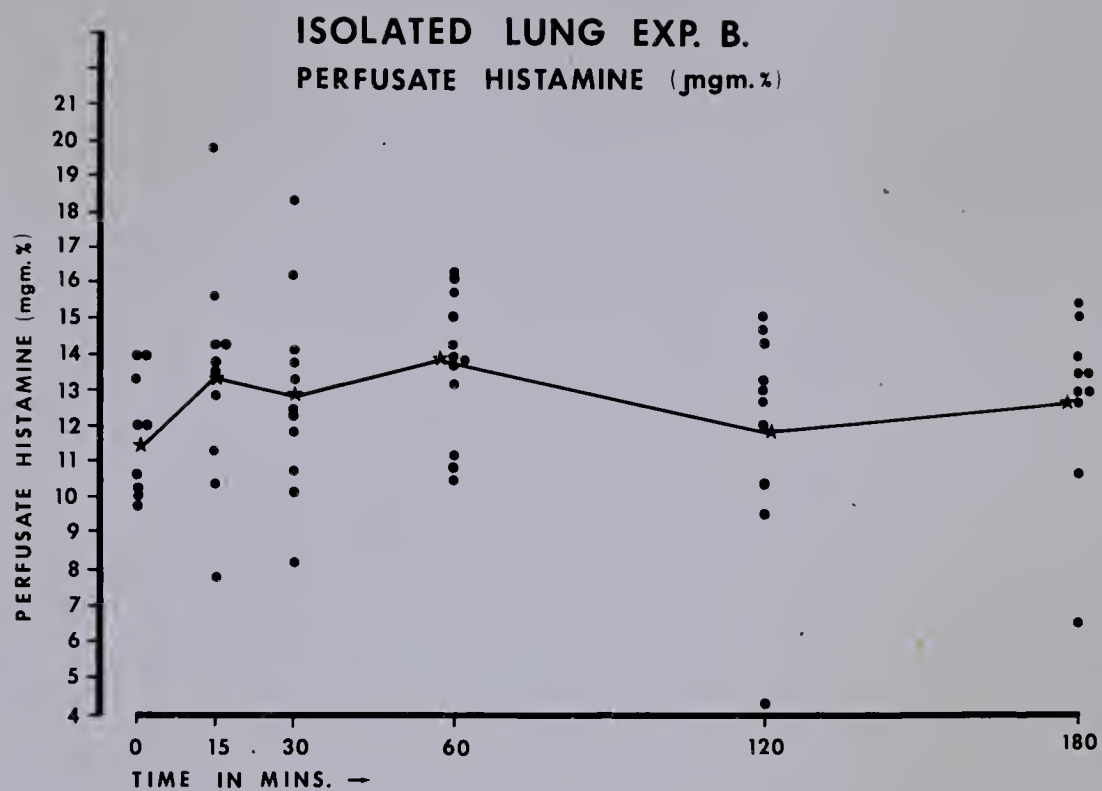


Subsidiary Figure 5

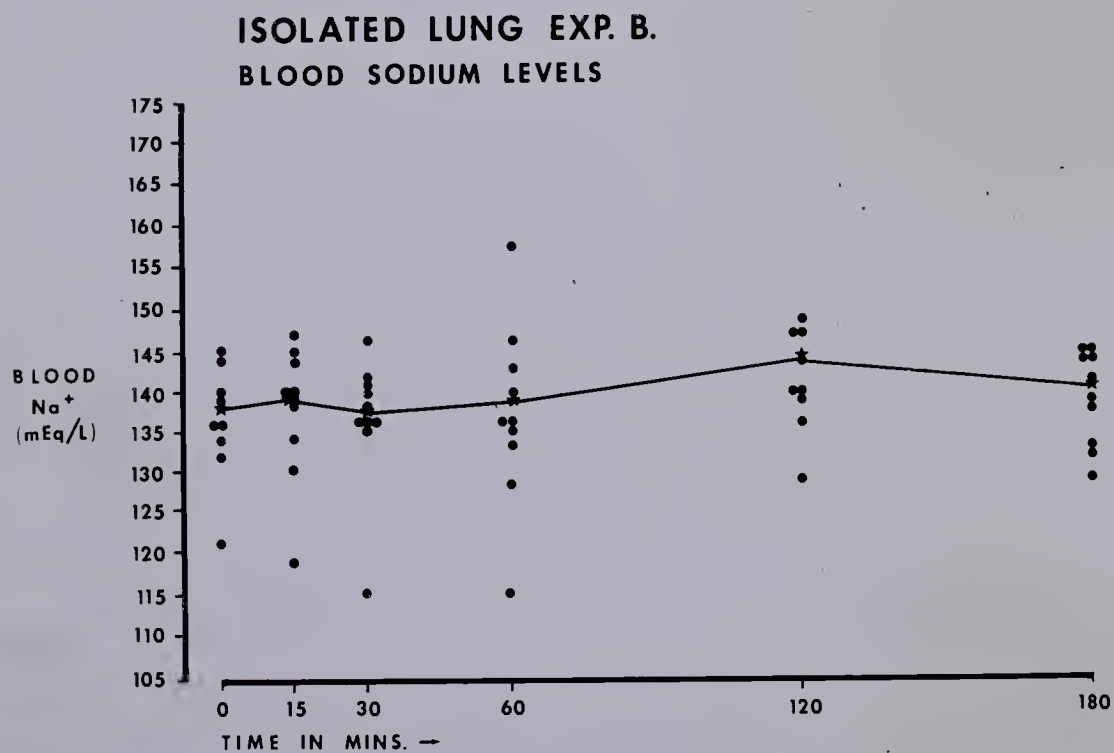


Subsidiary Figure 6



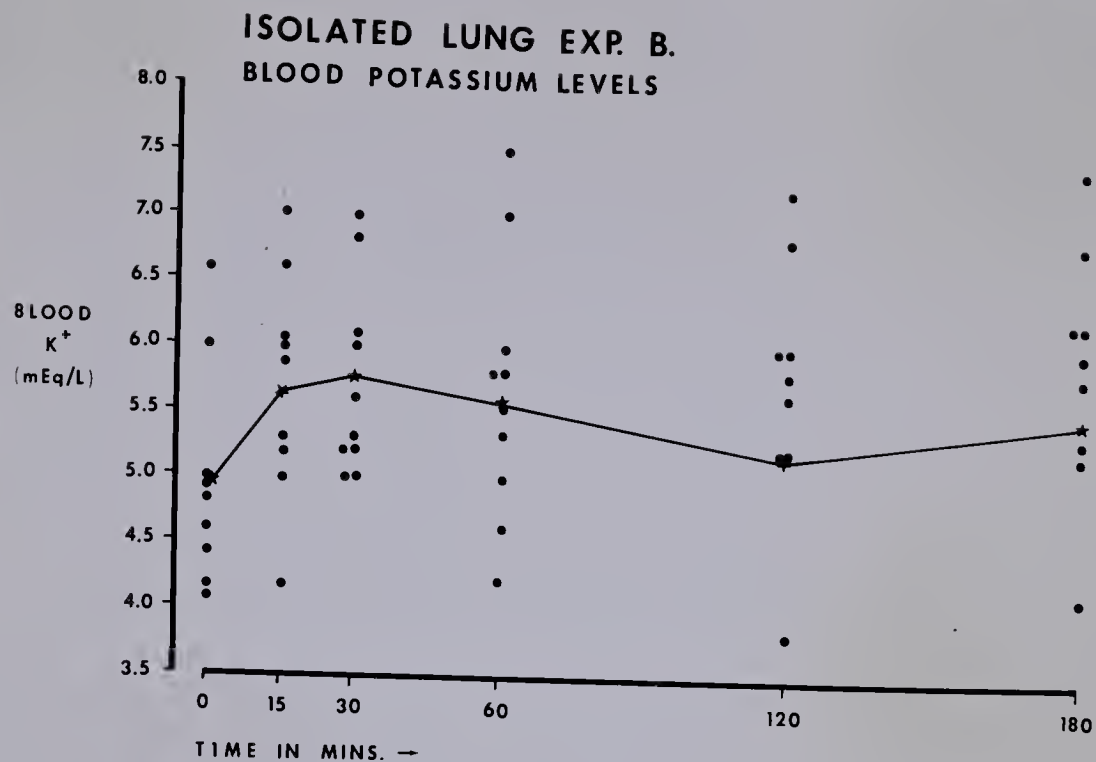


Addendum Figure 5.

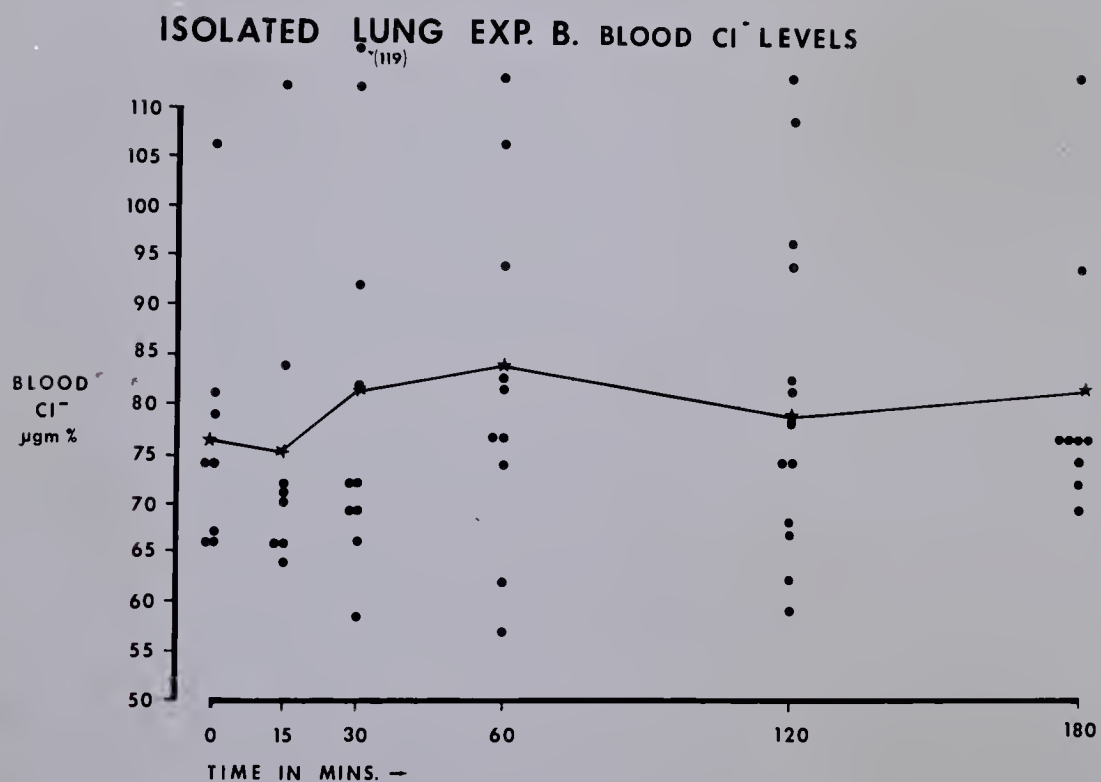


Addendum Figure 6.





Addendum Figure 7.

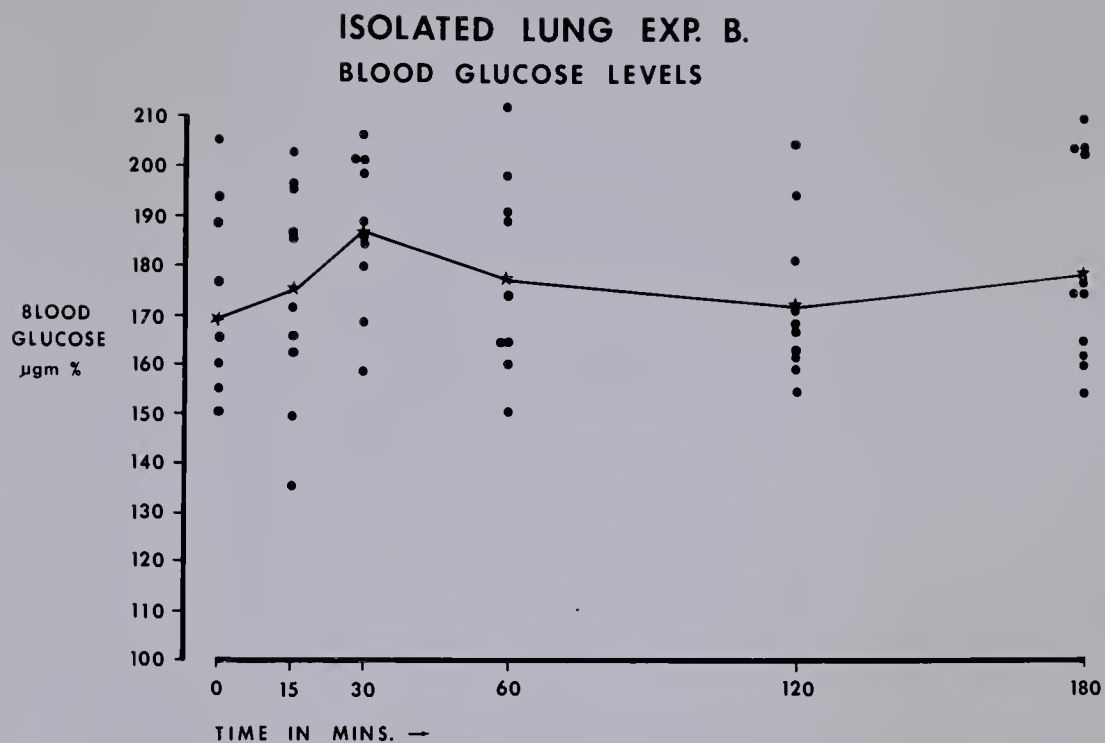


Addendum Figure 8.

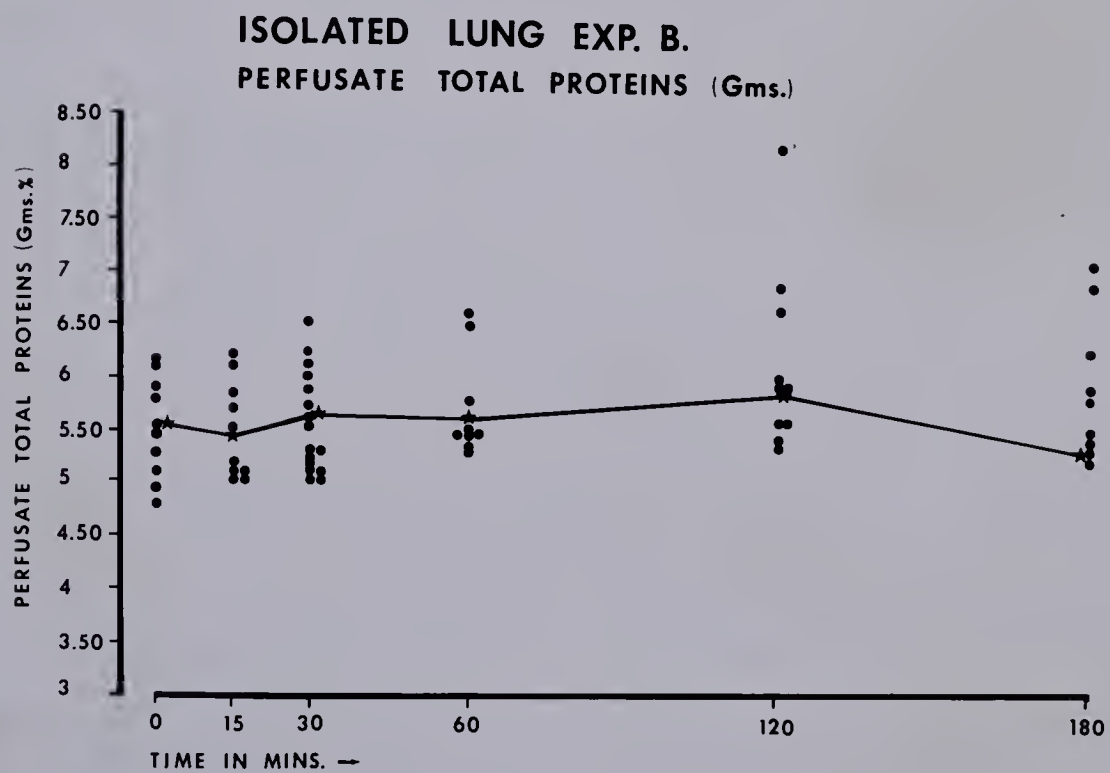


*Actitis macularia*





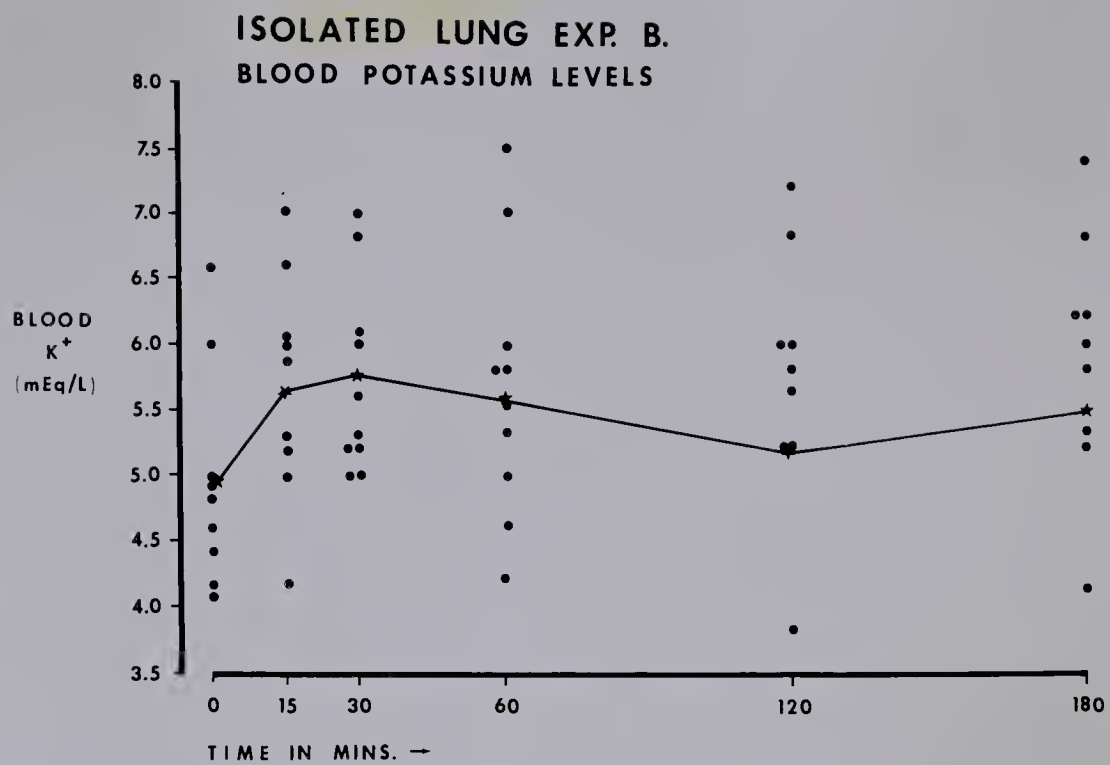
Addendum Figure 9.



Addendum Figure 10.







Addendum Figure 11.



# SAMPLE CALCULATION UTILIZING THE POOLED "T" TEST

## ISOLATED LUNG EXPERIMENT A

### POTASSIUM DETERMINATION DATA

$x_1$  = K at zero minutes perfusion time

$x_2$  = K at 180 minutes perfusion time

$$\Sigma x_1 = 40.30$$

$$\Sigma x_2 = 41.45$$

$$\Sigma x_1^2 = 183.59$$

$$\Sigma x_2^2 = 253.61$$

$$(\Sigma x_1)^2 = 1624.09$$

$$(\Sigma x_2)^2 = 1718.10$$

Where  $\Sigma x_1$  = the sum of the  $x_1$  variants  
 $\Sigma x_1^2$  = the sum of the squares of the  $x_1$  variants  
and  $(\Sigma x_1)^2$  = the square of the sum of the  $x_1$  variants

$$\Sigma x_1^2 = \frac{(\Sigma x_1)^2}{n} \quad \text{where } n = \text{the number of variants}$$

and  $S^2 = \frac{\Sigma x_1^2 + \Sigma x_2^2}{(n_1-1)+(n_2-1)}$  where  $S^2$  = the square of the variance of the sample  
and  $(n_1-1)$  = the degrees of freedom for  $x_1$  = df

When the criterion F is used, the results are shown in an analysis of variance table:

### ANALYSIS OF VARIANCE OF DATA

Source of Variation	df	SS sum of squares	mean square	F
Group A potassium zero minutes vs 180 minutes	1	A=8.20	$\frac{A}{1} = 8.20$	$\frac{8.20}{0.808} = 10.15$
Among Group A potassium zero minutes and among 180 minutes	14	B+C=11.31	$\frac{B+C}{df} = \frac{11.31}{14} = 0.808$	

# STATISTICS

CHAPTER 1

STATISTICS

STATISTICS

STATISTICS

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$$\text{Where: } A = \frac{(\sum X_1)^2}{n_1} + \frac{(\sum X_2)^2}{n_2} - \frac{(\sum X_1 + \sum x_2)^2}{n_1 + n_2}$$

$$B = \sum X_1^2 = \sum x_1^2 - \frac{(\sum x_1)^2}{n_1}$$

$$C = \sum X_2^2 = \sum x_2^2 - \frac{(\sum x_2)^2}{n_1}$$

Comparing the calculated F value with the tabulated F value for the proper degrees of freedom, if the calculated F value is higher than the tabulated one the difference between the variants is highly significant. In this instance the calculated F is greater than the tabulated F at the 1.0 to 0.51 level. That is, the chance of the existing relationship between the variants occurring by chance alone is only 1.0 to 0.5%. Therefore the difference in the variants is deemed highly significant.

$$E = \frac{1}{n} \sum_{i=1}^n (y_i - \hat{y}_i)^2 = \frac{1}{n} \sum_{i=1}^n (y_i - \bar{y})^2 + \frac{1}{n} \sum_{i=1}^n (\bar{y} - \hat{y}_i)^2$$

$$E = \frac{1}{n} \sum_{i=1}^n (y_i - \bar{y})^2 + \frac{1}{n} \sum_{i=1}^n (\bar{y} - \hat{y}_i)^2$$

$$E = \frac{1}{n} \sum_{i=1}^n (y_i - \bar{y})^2 + \frac{1}{n} \sum_{i=1}^n (\bar{y} - \hat{y}_i)^2$$

Comparing the estimated  $\hat{y}$  value with the observed  $y$  value for the proper degree of freedom. If the estimated  $y$  value is higher than the observed one the difference between the observed  $y$  and the estimated  $\hat{y}$  is highly significant. In this instance the estimated  $\hat{y}$  is greater than the observed  $y$  at the 0.05 level. That is, the chance of the resulting relationship between the variables occurring by chance alone is only 0.05. Therefore the difference in the variance is small. Hence significant.

R E F E R E N C E S

1. Hardy, J.D., The transplantation of organs, Surgery 56: 685, 1964.
2. Moore, F.D., Give and take, Philadelphia: Saunders, 1964, p.41.
3. Moore, F.D., Give and take, Philadelphia: Saunders, 1964, p.8-10.
4. Carrel, A., Results of transplantation of blood vessels, organs and limbs, J.A.M.A. 51: 1662, 1908.
5. Carrel, A., Transplantation in mass of the kidneys, J. Exp. Med., 10: 98, 1908.
6. Carrel, A. and Lindberg, C.A., The culture of organs, London: Hamish, Hamilton, 1938.
7. Guthrie, C.C., Some physiological aspects of blood vessel surgery, J.A.M.A., 51: 1658, 1908.
8. Moore, F.D., Give and take, Philadelphia: Saunders, 1964, p.47.
9. Moore, F.D., Give and take, Philadelphia: Saunders, 1964, p.49.
10. Billingham, R.E., Brent, L. and Medawar, P.B., Actively acquired tolerance of foreign cells, Nature, London 172: 603-606, Oct.3, 1953.
11. Moore, F.D., Give and take, Philadelphia: Saunders, 1964, p.59.
12. Woodruff, M.F.A., The transplantation of tissues and organs, Springfield, Thomas: 1960, p.3.
13. Woodruff, M.F.S., The transplantation of tissues and organs, Springfield, Thomas: 1960, p.210.
14. Woodruff, M.F.A., The transplantation of tissues and organs, Springfield, Thomas: 1960, p.517.

THE UNIVERSITY OF CHICAGO

1. The University of Chicago Press, Chicago, Ill., 1961.
2. The University of Chicago Press, Chicago, Ill., 1961.
3. The University of Chicago Press, Chicago, Ill., 1961.
4. The University of Chicago Press, Chicago, Ill., 1961.
5. The University of Chicago Press, Chicago, Ill., 1961.
6. The University of Chicago Press, Chicago, Ill., 1961.
7. The University of Chicago Press, Chicago, Ill., 1961.
8. The University of Chicago Press, Chicago, Ill., 1961.
9. The University of Chicago Press, Chicago, Ill., 1961.
10. The University of Chicago Press, Chicago, Ill., 1961.
11. The University of Chicago Press, Chicago, Ill., 1961.
12. The University of Chicago Press, Chicago, Ill., 1961.
13. The University of Chicago Press, Chicago, Ill., 1961.
14. The University of Chicago Press, Chicago, Ill., 1961.



15. Simonsen, M., Biological incompatibility in kidney transplantation in dogs. II. Serological investigations, Acta. Path. Microbiol. Scand., 32: 36, 1953.
16. Dempster, W.J., A consideration of the cause of functional arrest of homotransplanted kidneys, Brit. J. Urol., 27, 66, 1955.
17. Cauthorn, F.M., and Hardy, J.D., Transplantation of tissues, Amer. J. Med. Sci. 242: 246, 1961.
18. Woodruff, M.F.A., The transplantation of tissues and organs, Springfield, Thomas, 1960, p.518.
19. Woodruff, M.F.A., The transplantation of tissues and organs, Springfield, Thomas, 1960, p.68 et seq.
20. Raffel, S., Immunity - New York: Appleton, Century-crofts, 1960, p.549.
21. Merrill, J.P., Antigen and antibody in transplantation immunity: Some problems of investigations. In: Biological problems of grafting. Les Congres et colloques de l'Universite de Liege, Vol. 12, p.34, Liege, 1955.
22. Murray, J.E., Wilson, R.E., Realy, J.B., Sadowsky, N. and Carson, J., Skin grafts in irradiated rabbits treated with marrow from single and multiple donors. In: Biological problems in grafting. Les Congres et colloques de l'Universite de Liege, Vol.12, p.354, Liege, 1959.
23. Dempster, W.J., Kidney homotransplantation. Brit.J.Surg. 40: 447, 1953.
24. Baher, R. and Gordon, R., Effect of total body irradiation on experimental renal transplantation, Surgery 37: 820, 1955.
25. Binhammer, R.T., Schneider, M. and Finerty, S.C., Time as a factor in post irradiation protection by parabiosis, Amer. J. Physiol. 175: 440, 1953.
26. Calne, R.V., Renal transplantation. London, Arnold: p.100, 1963.
27. Calne, R.V., Renal transplantation. London, Arnold: p.157, 1963.



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- 2. The second is the fact that the...
- 3. The third is the fact that the...
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- 7. The seventh is the fact that the...
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28. Rowley, D.A., Effect of splenectomy on formation of circulating antibody in adult male albino rat. *J. Immunol.* 64: 289, 1950.
29. Billingham, R.E., Brent, L. and Medawar, P.B., Quantitative studies on tissue transplantation immunity, *Phil. Trans. B.* 239: 357, 1956.
30. Good, R.A., Progress toward transplantation in man., *Advances Pediat.*, 13: 93, 1964.
31. Rowley, D.A., The formation of circulating antibody in the splenectomized human being following intravenous injection of heterologous erythrocytes., *J. Immunol.* 65: 515, 1950.
32. Scothorne, R.J. and McGregor, I.A., Cellular changes in lymph nodes and spleen following skin homografting in the rabbit. *J. Anat.*, London, 89: 283, 1955.
33. Blumenthal, H.T., Effect of organismal differentials on the distribution of leukocytes in the circulating blood. *Arch. Path.*, 27: 510, 1939.
34. Blumenthal, H.T., Organismal differentials: Further investigations of their effect on distribution of leukocytes in circulating blood. *Arch. Path.*, 31: 295, 1941.
35. Rogers, B.O., Converse, J.M., Taylor, A.C. and Campbell, R.M., Eosinophils in human skin homografts. *Proc. Soc. Exp. Biol.*, N.Y., 82: 523, 1953.
36. Raffel, S., *Immunity.* New York, Appleton, Century-Crofts, 1960, p.553.
37. Snell, G.D., Methods for study of histocompatibility genes and isoantigens. *Meth. Med. Res.* 10: 1, 1964.
38. Raffel, S.: *Immunity.* New York, Appleton, Century-Crofts, 1960, p.553.
39. Landsteiner, K. and Wiener, A.A.: An agglutinable factor in human blood recognized by immune sera for blood. *Proc. Soc. Exp. Biol. Med.*, 43: 223, 1940.
40. Hume, D.M., Merrill, J.P., Miller, B.F. and Thorn, G.W.: Experiences with renal homotransplantation in the human. Report of nine cases. *J. Clin. Invest.*, 34: 327, 1955.

- 1. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 2. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 3. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 4. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 5. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 6. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 7. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 8. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 9. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 10. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 11. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 12. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 13. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 14. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 15. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 16. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 17. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 18. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 19. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.
- 20. *Journal of the Royal Society of Medicine*, 1911, 4, 1, 1-10.



41. Swan, H. and Rundles, W.R.: Islet cell transplantation in dogs. Transpl. Bull., 4, 53, 1957.
42. Zhordania, I.F. and Gotsiridze, O.A.: Vital activity of the excised uterus and its appendages after their autotransplantation into omentum. Experimental research. Acta. Chir. Plast. (Praha), 6: 23-32, 1964.
43. Hardy, J.D., Eraslan, S. and Webb, W.R.: Transplantation of the lung. Ann. Surg. 160: 440, 1964.
44. Hardy, J.D., Kumis, F.D. and Chavez, C.M.: Heart transplantation in man. Developmental studies and report of a case. J.A.M.A. 188: 1132, 1964.
45. Dhemikov, V.P.: Experimental transplantation of vital organs. New York, Consultants Bureau Enterprises, Inc., 1962.
46. Hardy, J.D.: Autotransplantation of adrenal remnants to thigh in Cushing's disease. J.A.M.A. 185: 134, 1963.
47. Hardy, J.D.: High ureteral injuries. Management by autotransplantation of the kidney. J.A.M.A. 184: 97, 1963.
48. Second Annual Human Kidney Transplant Conference - National Research Council - Washington, D.C. May, 1965.
49. Hardy, J.D., Webb, W.R., Dalton, M.L. Jr., and Walker, G.R., Jr.: Lung homotransplantation in man. J.A.M.A. 186: 1065, 1963.
50. Magovern, G.L. and Yates, A.J.: Human homotransplantation of the left lung. Report of a case. Ann. N.Y. Acad. Sci., 120: 710, 1964.
51. Hardy, J.D.: Personal communication. June, 1964.
52. Alican, F. and Hardy, J.D.: Lung reimplantation. J.A.M.A. 183: 849, 1963.
53. Amirana, M.T., Rohman, M., Oka, M., Kikkawa, Y., Gueft, B. and State, D.: Functional and pathologic changes in the reimplanted lung. Surg. Forum. 15: 177, 1964.
54. Archer, F.L., Gago, O., Delgado, E., Kolb, L.W. and Adams, W.E.: Histologic pattern in the auto- and homotransplanted dog lung. Surg. Forum 15: 154, 1964.





55. Ballinger, W.F., II., Scicchitano, L.P., Baranski, E.J. and Camishion, R.C.: The effects of cardiopulmonary denervation. *Surgery*, 55: 574, 1964.
56. Baranski, E.J., Scicchitano, L.P., Camishion, R.C. and Ballinger, W.F., II.: Pulmonary hypertension following cardiopulmonary transplantation. *Surg. Forum*, 14: 299, 1963.
57. Barnes, B.A. and Flax, M.R.: Experimental pulmonary homografts in the dog: II. Modification of the homograft response by BW 57.322. *Transplantation*, 2: 343, 1964.
58. Barnes, B.A., Flax, M.H., Burke, J.F. and Barr, G.: Experimental pulmonary homografts in the dog: I. Morphological studies. *Transplantation*, 1: 351, 1963.
59. Benfield, J.R., Gago, O., Nigro, S.L., Fry, W.A. and Adams, W.E.: Role of the adventitia of the pulmonary hilum as related to the transplantation problem. *Surg. Forum*, 14: 198, 1963.
60. Blades, B., Beattie, E.J., Jr., and Elias, W.S.: The surgical treatment of intractable asthma. *J. Thoracic Surg.*, 20: 584, 1950.
61. Blades, B., Beattie, E.J., Jr., Hill, R.P. and Thistlewaite, R.: Ischemia of the lungs. *Ann. Surg.*, 136: 56, 1952.
62. Blades, B., Pierpoint, H.C., Samadi, H. and Hill, R.P.: The effect of experimental lung ischemia on pulmonary function. *Surg. Forum*, 4: 255, 1953.
63. Blanco, G., Adam, A., Rodriguez-Perez, D. and Fernandez, A.: Complete homotransplantation of canine heart and lungs. *Arch. Surg.*, (Chicago), 76: 20, 1958.
64. Blumenstock, D.A., Collins, J.A., Hechtman, H.B., Hosbein, D.J., Thomas, E.D. and Ferrebee, J.W.: Late results of homotransplantation of the lung in dogs. *Dis. Chest*, 45: 484, 1964.
65. Blumenstock, D.A., Collins, J.A., Thomas, E.D. and Ferrebee, J.W.: Homotransplantation of the lung in dogs treated with methotrexate. *Surg. Forum*, 12: 121, 1961.
66. Blumenstock, D.A., Collins, J.A., Thomas, E.D. and Ferrebee, J.W.: Homotransplants of the lung in dogs. *Surgery*, 51: 541, 1962.
67. Blumenstock, D.A., Hechtman, H.B. and Collins, J.A.: Preservation of the canine lung. *J. Thoracic Cardiovasc. Surg.*, 44: 771, 1962.





68. Blumenstock, D.A. and Kahn, D.R.: Replantation and transplantation of the canine lung. *J. Surg. Res.*, 1: 40, 1961.
69. Bogardus, G.M.: An evaluation in dogs of the relationship of pulmonary bronchial, and hilar adventitial circulation to the problem of lung transplantation. *Surgery*, 43: 849, 1958.
70. Borrie, J. and Lichter, I.: Lung transplantation: Technical problems. *Thorax*, 19: 383, 1964.
71. Borrie, J. and Montgomerie, J.Z.: Lung excision and re-implantation in sheep. *Proc. Univ. Otago Med. Sch. (New Zealand)* 36: 9, 1958.
72. Buecherl, E.S., Lesch, P., Nasser, M.: Ergebnisse experimenteller Untersuchungen nach normothermer und hypothermer Homoiotransplantation einer Lunge. *Langenbeck Arch. Klin. Chir.*, 296: 660, 1961.
73. Buecherl, E.S., Nasser, M. and vonProndzynski, B.: Lung function studies after homotransplantation, autotransplantation, denervation of the left lung, and ligation of the right pulmonary artery. *J. Thorac. Cardiovasc. Surg.*, 47: 455, 1964.
74. Carter, M.G. and Strieder, J.W.: Resection of the trachea and bronchi: An experimental study. *J. Thorac. Surg.*, 20: 613, 1950.
75. Christiansen, K.H., Smith, D.E. and Pinch, L.W.: Homologous transplantation of canine lungs: Technique with contralateral pulmonary artery ligation. *Arch. Surg. (Chicago)*, 86: 495, 1963.
76. Davis, H.A., Gordon, W.B., Hayes, E.W., Jr. and Wasley, M.T.: Effects upon the lung of varying periods of temporary occlusion of the pulmonary artery. *Arch. Surg. (Chicago)*, 64: 464, 1952.
77. Davis, H.A., O'Connor, J.P., Coloviras, C.J., Jr., and Strawn, D.L.: Homologous transplantation of the lung. *Arch. Surg. (Chicago)*, 64: 745, 1952.
78. Debono, A.H.C. and Brock, R.C.: Lung transplantation. *Brit. J. Surg.*, 51: 72, 1964.
79. Duvoisin, G.E., Fowler, W.S., Paynes, W.S. and Ellis, F.H. Jr.: Reimplantation of the dog lung with survival after contralateral pneumonectomy. *Surg. Forum*, 15: 173, 1964.



80. Ellis, E.E. and Richards, V.: The fate of homologous lung transplants in dogs. *Surgery*, 36: 1109, 1954.
81. Ellis, F.H. Jr., Grindlay, J.H. and Edwards, J.E.: The bronchial arteries: I. Experimental occlusion. *Surgery*, 30: 810, 1951.
82. Ellison, L.T., Yeh, T.J. and Ellison, R.G.: Cardiopulmonary physiology following lung reimplantation. *Fed. Proc.* 21: 438, 1962.
83. Eraslan, S., Turner, M.D. and Hardy, J.D.: Lymphatic regeneration following lung reimplantation in dogs. *Surgery*, 56: 970, 1964.
84. Faber, L.P. and Beattie, E.J. Jr.: Respirations following lung denervation. *Surg. Forum*, 9: 383, 1958.
85. Faber, L.P., Kenwell, J.M. and Beattie, E.J. Jr.: Homologous lung transplantation: Experiences in the dog. *Arch. Surg. (Chicago)*, 83: 491, 1961.
86. Fegiz, G., Leggeri, A., Blasucci, E., and Drago, G.: Studio sperimentale sul trapianto polmonare con particolare riguardo all'impiego del raffreddamento rapido quale tentativo per ottenere l'attecchimento definitivo. *Arch. Chir. Torace*, 16: 539, 1959.
87. Gago, O., Delgado, E., Benfield, J.R., Nigro, S.L., Ranninger, K. and Adams, W.E.: Left lower pulmonary lobe homotransplantation. *J.A.M.A.* 188: 443 (abstract), 1964.
88. Haglin, J.J. and Orn, A.: Physiologic studies of the baboon living on only the reimplanted lung. *Surg. Forum*, 15: 175, 1964.
89. Haglin, J., Telander, R.L., Muzzall, R.E., Kiser, J.C. and Strobel, C.J.: Comparison of lung autotransplantation in the primate and the dog. *Surg. Forum*, 14: 196, 1963.
90. Hankinson, H.W. and Edwards, F.R.: The effect of pulmonary ischemia on lung function. *Thorax*, 14: 122, 1959.
91. Hardin, C.A. and Kittle, C.F.: Experiences with transplantation of the lung. *Science*, 119: 97, 1954.
92. Hardin, C.A., Kittle, C.F. and Schafer, P.W.: Preliminary observations on homologous lung transplants in dogs. *Surg. Forum*, 3: 374, 1952.





93. Hardy, J.D., Eraslan, S. and Dalton, M.L. Jr.: Autotransplantation and homotransplantation of the lung: Further studies. J. Thorac. Cardio. Surg., 46: 606, 1963.
94. Hardy, J.D., Eraslan, S., Dalton, M.L. Jr., Alican, F. and Turner, M.D.: Reimplantation and homotransplantation of the lung: Laboratory studies and clinical potential. Ann. Surg., 157: 707, 1963.
95. Hechtman, H.B., Blumenstock, D.A., Thomas, E.D. and Ferrebee, J.W.: Organ transplants in dogs after cross-circulation, chemotherapy, and radiation. Surgery, 52: 810, 1962.
96. Howard, H.S. and Webb, W.R.: Respiratory paralysis following pulmonary denervation. Surg. Forum, 8: 466, 1957.
97. Hughes, F.A., Kehne, J.H. and Fox, J.R.: Replantation and transplantation of pulmonary tissue in dogs. Surgery, 36: 1101, 1954.
98. Jackson, T.L., Lefkin, P., Tuttle, W. and Hampton, F.: An experimental study of bronchial anastomosis. J. Thorac. Surg., 18: 630, 1949.
99. Lanari, A., Croxatto, O.C. and Molins, M.: Autoinjerto de pulmon en perros. Medicina (B. Air), 15: 83, 1955.
100. Lempert, N. and Blumenstock, D.A.: Survival of dogs after bilateral reimplantation of the lungs. Surg. Forum, 15: 179, 1964.
101. Lindberg, E.J., Demetriades, A., Armstrong, B.W. and Konsuwan, N.: Lung reimplantation in the dog. J.A.M.A. 178: 486, 1961.
102. Lower, R.R., Stofer, R.C., Hurley, E.J. and Shumway, N.E.: Complete homograft replacement of the heart and both lungs. Surgery, 50: 842, 1961.
103. MacPhee, I.W. and Wright, E.S.: Experimental lung transplantation. Lancet, 1: 192, 1964.
104. Metras, H.: Note preliminaire sur la greffe totale du poumon chaz le chien. C.R. Acad. Sci. (Paris), 23: 1176, 1950.
105. Neptune, W.B., Cookson, B.A., Bailey, C.P., Appler, R. and Rajkowski, F.: Complete homologous heart transplantation. Arch. Surg. (Chicago) 66: 174, 1953.





106. Neptune, W.B., Redondo, H. and Bailey, C.P.: Experimental lung transplantation. Surg., Forum, 3: 379, 1952.
107. Neptune, W.B., Redondo, H. and Bailey, C.P.: Experimental lung transplantation. J. Thorac. Surg., 26: 275, 1953.
108. Nigro, S.L., Evans, R.H., Benfield, J.R., Gago, O., Fry, W.A. and Adams, W.E.: Physiological alterations of cardiopulmonary function in dogs living one and one-half years on only a reimplanted right lung. J. Thorac. Cardio. Surg., 46: 598, 1963.
109. Nigro, S.L., Evans, R.H., Gago, O. and Adams, W.E.: Altered physiology of the pulmonary vascular bed: A factor in decreased function of the reimplanted lung. Dis. Chest, 46: 318, 1964.
110. Nigro, S.L., Reiman, A.F., Fry, W.A., Mock, L.F. and Adams, W.E.: Alterations in cardiopulmonary physiology following autotransplantation of the lung. Surg. Forum, 12: 56, 1961.
111. Nigro, S.L., Reimann, A.F., Mock, L.F., Fry, W.A., Benfield, J.R. and Adams, W.E.: Dogs surviving with a reimplanted lung. J.A.M.A. 183: 854, 1963.
112. Ota, Y., Camishion, R.C. and Gibbon, J.H.: *Dirofilaria immitis* (heart worms) and *Dipetalonema* species as causes of "transfusion reaction" in dogs. Surgery, 51: 518, 1962.
113. Parsa, P., Faber, L.P., Staub, E.W. and Beattie, E.J. Jr.: Experimental homotransplantations of the lungs with cytotoxic agents. Dis. Chest, 45: 365, 1964.
114. Portin, B.A., Rasmussen, G.L., Stewart, J.D. and Andersen, M.N.: Physiologic and anatomic studies thirty-five months after successful replantation of the lung. J. Thorac. Cardio. Surg., 39: 380, 1960.
115. Richter, M. and Buecherl, E.S.: Pathologisch-anatomische und funktionelle Veränderungen an der transplantierten Lunge. Verh. Deutsch. Ges. Path., 44: 178, 1960.
116. Slim, M.S., Yacoubain, H.D., Wilson, J.L., Rubeiz, G.A. and Ghandur-Manymneh, L.: Successful bilateral reimplantation of canine lungs. Surgery, 55: 676, 1964.
117. Swan, H. and Mulligan, R.M.: An experimental study of the effect of ligation of pulmonary veins in the dog. J. Thorac. Surg., 17: 44, 1948.





118. Trummer, M.J.: An improved tracheal divider for simultaneous bronchspirometry in the dog. *Surgery*, 56: 574, 1964.
119. Trummer, M.J.: Transplantation of the lung. *Annals of Thoracic Surgery*, 10: 144, 1965.
120. Trummer, M.J. and Christiansen, K.H.: Radiographic and functional changes following autotransplantation of the lung. To be published.
121. Waldhausen, J.A., Kilman, J.W. and Giammona, S.T.: Effect of autotransplantation and homotransplantation of the canine lung on pulmonary compliance and surfactant. *J.A.M.A.* 188: 440 (abstract), 1964.
122. Webb, W.R., deGuzman, V. and Hoopes, J.E.: Cardiopulmonary transplantation: Experimental study of current problems. *Amer. Surg.*, 27: 236, 1961.
123. Webb, W.R. and Howard, H.S.: Extension of the limits of cardiac viability with total coronary occlusion. *Surgery*, 42: 92, 1957.
124. Webb, W.R. and Howard, H.S.: Cardiopulmonary transplantation. *Surg. Forum.*, 8: 313, 1957.
125. Yeh, T.J., Ellison, L.T. and Ellison, R.G.: Functional evaluation of the autotransplanted lung in the dog. *Amer. Rev. Resp. Dis.*, 86: 791, 1962.
126. Zorzoli, G., Gualtieri, V., Serra, B. and Bergamini, D.: Etudes sur la reimplantation et la transplantation du poumon. *Bronches* 8: 354, 1958.
127. Shaw, K.M. and Burton, N.A.: Experimental pulmonary reimplantation. *Thorax*, 19: 180, 1964.
128. Reemtsma, K., Rogers, R.E., Lucas, J.F., Schmidt, F.E. and Davis, F.H.: Studies of pulmonary function in transplantation of the canine lung. *J. Thorac. Cardio. Surg.*, 46: 589, 1963.
129. Human Kidney Transplantation Conference, American College of Surgeons, Meeting, Chicago, October 1964.
130. Spectacular Problems in Surgery, American College of Surgeons Meeting, Chicago, October 1964. Film presented by Dr. J. D. Hardy.
131. Woodruff, M.F.A.: The transplantation of tissues and organs. Springfield, Thomas, p.24.



132. Woodruff, M.F.A.: The transplantation of tissues and organs. Springfield, Thomas, p.195., 1960.
133. Staudacher, V.E., Bellinazzo, P. and Pulin, A.: Primi-rilievi su tentativi di reimplanti autoplatici e di trapianti omoplastici di lobi polmonari. Chirurgia (Milano), 5: 223, 1950.
134. Juvenelle, A.A., Citret, C., Wiles, C.E. Jr. and Stewart, J.D.: Pneumonectomy with replantation of the lung in the dog for physiologic study. J. Thorac. Surg., 21: 111, 1951.
135. Blumenstock, D.A., Collins, J.A., Hechtman, H.B., Thomas, E.D. and Ferrebee, J.W.: Functioning homo-grafts of the lung in dogs. Ann. N.Y. Acad. Sci., 99: 882, 1962.
136. Widdecoombe, F.G.: Respiratory reflexes. In: Handbook of Physiology, section 3, volume 1: Respiration. Baltimore, Williams and Wilkins, 1964, p. 611.
137. Huggins, C.E.: Reimplantation of lobes of the lung: An experimental technique. Lancet 2: 1059, 1959.
138. Britt, C.I., Miller, E.M. Jr., Felder, M.E. and Sirak, H.D.: Comparative reaction of Mersilene and silk sutures implanted within the heart. Ann. Surg., 153: 52, 1961.
139. Rahn, H. and Farhi, L.E.: Ventilation, perfusion and gas exchange. In: Handbook of Physiology, section 3, volume 1: Respiration. Baltimore, Williams and Wilkins, 1964, p. 749.
140. Hardin, C.A.: Common antigenicity between skin grafts and total lung transplants. Surg. Forum, 7: 565, 1956.
141. Woodruff, M.F.A.: The transplantation of tissues and organs. Thomas, Springfield, 1960, p.159.
142. Woodruff, M.F.A.: The transplantation of tissues and organs. Thomas, Springfield, 1960, p. 155.
143. Luyet, B.J. and Hodapp, E.L.: Revival of frog spermat-ozoa vitrified in liquid air. Proc. Soc. Exp. Biol., N.Y., 39: 433, 1938.
144. Luyet, B.J. and Gehenio, P.M.: Life and death at low temperatures. Normandy (Miss.) Biodynamica, 1940. Quoted in Woodruff.



- 1. *Journal of the American Medical Association*, 1934, 103, 1-10.
- 2. *Journal of the American Medical Association*, 1934, 103, 11-20.
- 3. *Journal of the American Medical Association*, 1934, 103, 21-30.
- 4. *Journal of the American Medical Association*, 1934, 103, 31-40.
- 5. *Journal of the American Medical Association*, 1934, 103, 41-50.
- 6. *Journal of the American Medical Association*, 1934, 103, 51-60.
- 7. *Journal of the American Medical Association*, 1934, 103, 61-70.
- 8. *Journal of the American Medical Association*, 1934, 103, 71-80.
- 9. *Journal of the American Medical Association*, 1934, 103, 81-90.
- 10. *Journal of the American Medical Association*, 1934, 103, 91-100.

145. Luyet, B.J. and Hartung, M.C.: Factors in revival of *Anquillula aceti* after its solidification in liquid air. *Amer. J. Physiol.*, 133: 368, 1941.
146. Lovelock, J.E.: The mechanism of protective action of glycerol against hemolysis by freezing and thawing. *Biochem. Biophys. Acta.*, 11: 28, 1953.
147. Lovelock, J.E.: Biophysical aspects of freezing. In: *Preservation and transplantation of normal tissues*. Ciba Foundation Symposium, p. 131. London: Churchill, 1954.
148. Lovelock, J.E.: Denaturation of lipid-protein complexes as a cause of damage by freezing. *Proc. Roy. Soc. B.*, 147: 427, 1957.
149. Lovelock, J.E.: Haemolysis by thermal shock. *Brit. J. Haematol.*, 1: 117, 1955.
150. Woodruff, M.F.A.: *The transplantation of tissues and organs*. Springfield, Thomas, 1960, p.162.
151. Barlyn, L.W., Berggren, R.B. and Lehr, H.B.: Frozen skin autografts protected by dimethyl sulfoxide. *Surg. Forum*, 15: 475, 1964.
152. Brown, H., Patel, J., Barsamian, E.M., Collins, S.C. and McDermott, W.V.: Cold preservation of liver for homotransplantation. *Surg. Forum*, 15: 215, 1964.
153. Smith, A.V.: Problems in the resuscitation of mammals from body temperatures below 0°C. *Proc. Roy. Soc. B.*, 147: 533, 1957.
154. Woodruff, M.F.A.: *The transplantation of tissues and organs*. Springfield, Thomas, 1960, p.181.
155. Aird, I.: In Melrose, D.G.: A mechanical heart-lung for use in man. *Brit. Med. J.*, 2: 57, 1953.
156. Lewis, F.J.: Hypothermia, Physiology and Clinical application. *Surg. Clin. N.A.*, 42: 69, 1962.
157. Markland, C. and Parson, F.M.: Preservation of kidneys for homotransplantation. *Brit. J. Urol.*, 35: 457, 1963.
158. Adolph, E.F.: Oxygen consumption of hypothermic rats and acclimatization to cold. *Amer. J. Physiol.*, 166: 75, 1951.





159. Bigelow, W.G. and Callaghan, J.C.: General hypothermia for experimental cardiac surgery. *Ann. Surg.*, 132: 531, 1950.
160. Hegnauer, A.H. and D'Amato, H.: Oxygen consumption and cardiac output in the hypothermic dog. *Amer. J. Physiol.*, 178: 138, 1954.
161. Kameya, S., Oz, M., Neville, W.E. and Clowes, G.H.Jr.: A study of oxygen consumption during profound hypothermia induced by perfusion of entire body. *S. Forum*, 11: 190, 1960.
162. Smith, A.V.: Biological effects of freezing and super-cooling. London, Edward Arnold Limited, 1961.
163. Levy, M.N.: Oxygen consumption and blood flow in hypothermic perfused kidney. *Amer. J. Physiol.*, 197: 1111, 1959.
164. Bogardus, G.M. and Schlosser, R.J.: Influence of temperature on ischemic renal damage. *Surgery*, 39: 970, 1956.
165. Mitchell, R.M. and Woodruff, M.F.A.: Renal cooling and ischemia. *Brit. J. Surg.*, 46: 593, 1959.
166. Schloerb, P.R., Waldorf, R.D. and Welsh, J.S.: Protective effect of kidney hypothermia on total renal ischemia. *Surg. Forum*, 8: 633, 1957.
167. Steuber, P., Kovacs, S., Koletsky, S. and Persky, L.: Regional renal hypothermia. *Surgery*, 44: 77, 1958.
168. Semb, C.: Partial resection of the kidney, anatomical, physiological and clinical aspects. *Ann. Roy. Coll. Surg. England*, 19: 137, 1956.
169. Mitchell, R.M.: Renal cooling and ischemia. *Brit. J. Surg.*, 46: 593, 1959.
170. Kovacs, S.: Protective value of hypothermia in renal anoxia. Evaluation of selective cooling of the kidney. *Anesth. Analg.*, 38: 157, 1959.
171. Semb, G., Krog, J. and Johansen, K.: Renal metabolism and blood flow during local hypothermia, studied by means of renal perfusion in situ. *Acta Chir. Scand. Suppl.*, 253: 196, 1960.
172. Kerr, W.K., Kyle, V.N., Kereskei, A.G. and Smythe, C.A.: Renal hypothermia. *J. Urol.*, 84: 236, 1960.





173. Dottorri, O., Ekestrom, S. and Hansson, L.O.: Local cooling of the kidney using perfusion technique. *Acta Chir. Scand.*, 124: 80, 1962.
174. Harvey, R.B.: Effect of temperature on function of isolated dog kidney. *Amer. J. Physiol.*, 197: 181, 1959.
175. Gelin, L.E. and Lofstrom, B.: A preliminary study on peripheral circulation during deep hypothermia. *Acta Chir. Scand.*, 108: 402, 1954.
176. Lynch, H.F. and Adolph, E.F.: Blood flow in small blood vessels during deep hypothermia. *J. Appl. Physiol.*, 11: 192, 1957.
177. Kristiansen, K. Selective cooling of organs with special reference to the brain. *J. Roy. Coll. Surg., Edin.*, 7: 1, 1961.
178. Kao, F.F. and Schlig, B.B.: Impairment of gas transport and gas exchange in dogs during acute hypothermia. *J. Appl. Physiol.*, 9: 387, 1956.
179. Gray, J.S.: Pulmonary ventilation and its physiological regulation. Springfield, Ill., Thomas 1950. Quoted in Kao (see supra).
180. Tanaka, T., Inoue, T. and Paton, B.C.: Oxygen availability during hypothermic perfusion using diluted blood. *S. Forum*, 13: 138, 1962.
181. Mallette, W.G., Fitzgerald, J.B. and Eiseman, B.: Hypercapnia - a means of increasing oxygen availability during hypothermic perfusion. *S. Forum*, 12: 184, 1961.
182. Bradley, A.F., Stupfel, M. and Severinghaus, J.W.: Effect of temperature on PCO<sub>2</sub> and PO<sub>2</sub> of blood in vitro. *J. Appl. Physiol.*, 9: 201, 1956.
183. Long, D.M., Folkman, M.J., Neptune, E.M. and Sudduth, H.C.: Pulmonary airway changes resulting from ischemia of the pulmonary artery. *S. Forum*, 13: 164, 1962.
184. Lapchinski, A.G.: Recent results of preserved limbs and kidneys and possible use of this technique in practice. *Ann. N.Y. Acad. Sci.*, 87: 539, 1960.
185. Knight, P.R., Tomkiewicz, Z.M. and Couch, N.P.: Evaluation of functioning canine renal autografts after six hours storage. *S. Forum*, 14: 171, 1963.





186. Cassie, G.F., Couch, N.P., Dammin, G.T. and Murray, J.E.: Normothermic perfusion and reimplantation of the excised dog kidney. *Surg. Gyn. Obst.*, 109: 721, 1959.
187. Couch, N.P., Cassie, G.F. and Murray, J.E.: Survival of the excised dog kidney perfused in a pump oxygenator system. *Surgery*, 44: 666, 1958.
188. Starling, E.H. and Verney, E.B.: The secretion of urine as studies on the isolated kidney. *Proc. R. Soc., Ser. B. Biol. Sc.*, London, 97: 321, 1925.
189. Hemingway, A.: Some observations on perfusion of isolated kidney by pump. *J. Physiol.*, London, 71: 201, 1931.
190. Crisp, N.W., Campbell, G.S. and Brown, E.B. Jr.: Studies on perfusion of human blood through the isolated dog lung. *S. Forum*, 6: 286, 1955.
191. Campbell, G.S., Crisp, N.W. and Brown, E.B. Jr.: Maintenance of respiratory function with isolated lung lobes during cardiac inflow occlusion. *Proc. Soc. Exp. Biol.*, 88: 390, 1955.
192. Riley, R.L., Cournand, A. and Donald, K.W.: Analysis of factors affecting partial pressures of oxygen and carbon dioxide in gas and blood of lungs. *Methods. Amer. J. Appl. Physiol.*, 4: 102, 1951.
193. Krah1, V.E.: Anatomy of the Mammalian Lung. In: *Handbook of Physiology, Section 3, Volume 1: Respiration*, Baltimore: Williams and Wilkins, 1964.
194. Duke, H. and Lee, G.deJ.: The regulation of blood flow through the lungs. *Brit. Med. Bull.*, 19: 71, 1963.
195. Bannister, J. and Torrance, R.W.: The effects of the tracheal pressure upon flow: Pressure relations in the vascular bed of isolated lungs. *Quart. J. Exp. Physiol.* 45: 352, 1960.
196. Wesolowski, S.A., Fisher, J.H. and Welch, C.S.: Heart-lung bypass using pumps and isolated homologous lungs. *Surg. Gynec. Obst.*, 95: 762, 1952.
197. Mustard, W.T. and Chute, A.L.: A surgical approach to transposition of the great vessels with extra-corporeal circuit. *Surgery*, 36: 39, 1954.





198. Markland, C. and Parson, F.M.: Preservation of kidneys for homotransplantation. *Brit. J. Urol.*, 35: 457, 1963.
199. Telander, R.L.: Prolonged normothermic perfusion of the isolated primate and sheep kidney. *Surg. Gynec. Obst.*, 118: 347, 1964.
200. Rosenfeld, S., Sellers, A.L. and Katz, J.: Development of an isolated perfused mammalian kidney. *Amer. J. Physiol.*, 196: 1155, 1959.
201. Pierpont, H.: Techniques of lung perfusion with cancer chemotherapeutic agents. *Cancer Chemo. Rep.*, 10: 15, 1960.
202. Jacobs, J.K., Flexner, J.M. and Scott, H.W. Jr.: Selective isolated perfusion of the right or left lung. *J. Thor. Cardiovasc. Surg.*, 42: 546, 1961.
203. Hyman, M.M. and Harris, M.S.: Isolation perfusion of nitrogen mustard as applied to the dog lung. *Cancer Chemo. Rep.*, 16: 519, 1962.
204. Pierpont, H. and Blades, B.: Lung perfusion with chemotherapeutic agents. *J. Thor. Cardiovasc. Surg.*, 39: 159, 1960.
205. Permutt, S., Bromberger-Barnes, B. and Bane, H.N.: Alveolar pressure, pulmonary venous pressure, and the vascular waterfall. *Med. Thorac.*, 19: 239, 1962.
206. Sjostrand, T.: Regulation of blood distribution in man. *Acta Physiol. Scand.*, 26: 312, 1952-53.
207. Lee, G. deJ. and DuBois, A.B.: Pulmonary capillary blood flow in man. *J. Clin. Invest.* 34: 1380, 1955.
208. Prinzmetal, M., Ornitz, E.M. Jr., Simkin, B., and Bergman, H.C.: Arteriovenous anastomoses in liver, spleen, and lungs. *Am. J. Physiol.*, 152: 48, 1948.
209. Aurebeck-Lindseth, G., Kawk, H., Lindseth, E.O. and Cowley, R.A.: Studies on the pulmonary arteriovenous blood flow through channels larger than 20 plus or minus 3 microns. *Surg. Forum*, 13: 147, 1962.
210. Daly, I. deB.: Intrinsic mechanism of the lung. *Quart. J. Exp. Physiol.*, 43: 2, 1958.
211. Daly, I. deB. and McDougall, R.J.S.: The control of the circulation of the blood. London: W. Dawson, 1956.





212. Awad, J.E., Lemieux, J.M. and Beaulieu, M.: The technique for the perfusion of prolonged periods. C.M.A.J., 8: 100, 1965.
213. Hyperbaric oxygenation: Potentialities and problems. Report of the ad hoc committee on hyperbaric oxygenation, National Academy of Sciences - National Research Council, Washington, D.C., 1963.
214. Zaroff, L.I., Lowenstein, E., Walker, H.L. and Villarreal, V.: Excess lactate in cyanotic dogs during hyperbaric oxygenation. Surg. Forum., 15: 202, 1964.
215. Manax, W.G., Eyal, Z., Bloch, J.H., Largiacter, F., Hildago, F. and Lillehei, R.C.: Prolonged in vitro preservation of whole organs. An exhibit prepared for the 50th Clinical Congress of the American College of Surgeons, Chicago, October 5-9th, 1964.
216. Manax, W.G., Eyal, Z., Bloch, J.H., Largiacter, F., Hildago, F. and Lillehei, R.C.: Successful 24-hour in vitro preservation of canine kidneys by the combined use of hyperbaric oxygenation and hypothermia. Surgery, 56: 275, 1964.
217. Polezal, V.: The effect of long lasting oxygen inhalation upon respiratory parameters in man. Physiol. Bohemo-Slovenica, 11: 149, 1962. Quoted in: Hyperbaric oxygenation: potentialities and problems, see ref 214.
218. Ohlsson, W.T.L.: A study on oxygen toxicity at atmospheric pressure. Acta. Med. Scand. Suppl. 190: 1, 1947.
219. Smith, F.J.C., Bennett, G.A., Heim, J.W., Thomson, R.M. and Drinker, C.K.: Morphological changes in the lungs of rats living under compressed air conditions. J. Exp. Med., 56: 79, 1932.
220. Penrod, K.E.: Nature of pulmonary damage produced by high oxygen pressures. J. App. Physiol., 9: 1, 1956.
221. Comroe, J.H., Foster, R.E. II., Dubois, A.B., Briscoe, W.A., and Carlsen, E.: The lung. Chicago: Year Book Med. Pub., p. 353., 1962.
222. Clements, J.A.: Physico-chemical properties of pulmonary surface films in XXII Inter. Conj. of physiol. Science symposia and special lectures. Leiden. Intern. Conj. Physiol. Sci. Volume I. 1962, pp.268-274.





- 223. Mead, J., Whittenberger, J.L.: Handbook of Physiology, section 3: Respiration, Volume I, Am. Physiol. Soc., Washington, 1964, p.484.
- 224. Ed. Lancet. Oxygen under pressure. 7398: 1257, 1965.
- 225. Gable, W.D. and Townsend, F.M.: Lung morphology of individuals exposed to prolonged intermittent supplemental oxygen. Aerospace Med., 33: 1343, 1962.
- 226. Goodman, L.S. and Gilman, A.: The pharmacological basis of therapeutics. New York: McMillan, 1958, p. 131.
- 227. Rahn, H. and Fayhi, L.E.: Ventilation perfusion and gas exchange - the VA/Q concept. In: Handbook of physiology, section 3 - respiration, Volume 1. Baltimore: Williams and Wilkins, 1964, pp 735 et seq.
- 228. Rahn, H. and Fayhi, L.E.: Ventilation, perfusion and gas exchange - the VA/Q concept. In: Handbook of physiology, section 3 - respiration, Volume 1. Baltimore: Williams and Wilkins, 1964, p. 735.
- 229. Ulmer, W.T. and Reichel, G. Quoted by Rahn and Fayhi (see supra), p. 735.
- 230. Comroe, J.H., Forster, R.E. II., Dubois, A.B., Briscoe, W.A., Carlsen, E.: The lung, Chicago: Yearbook, p.72., 1962.
- 231. Howard, J.L.: Elevation of the pulmonary artery pressure due to gas embolus. Aerospace Med., 1964, 2: p. 110.
- 232. Schmidt, N.: Surgical-Medical Research Institute, University of Alberta, Edmonton, unpublished data.
- 233. Ruch, T.C. and Fulton, J.F.: Medical Physiology and biophysics. Saunders: Philadelphia, 1961, p. 706.
- 234. Ciba Symposium Foundation on Histamine Ea. G.E.W. Wolstenholme and O'Connor, C.M., London: Churchill, 1956.
- 235. Bliss, J.Q., and Walker, J.D.: Histamine release by homologous plasma in the dog. C.J. Bio. Physiol., 37: 371, 1959.
- 236. Ruch, T.C. and Fulton, J.F.: Medical Physiology and Biophysics. Saunders, Philadelphia, 1961, p 524.





237. Thomas, L.J. Jr., Griffo, Z.J. and Roos, A.: Quoted by Mead, J. and Wittenberger, J.L. in Handbook of Physiology, section 3, Respiration, Volume I. Baltimore, Williams and Wilkins, 1964.
238. Woodbury, R.A. and Hamilton, W.F.: The effects of histamine on the pulmonary blood pressure of various animals with and without anesthesia. J. Pharmacol. Exp. Therap., 71: 293, 1941.
239. Ruch, T.C. and Fulton, J.F.: Medical Physiology and Biophysics, Saunders: Philadelphia, 1961. pp.3-7.
240. Ruch, T.C. and Fulton, J.F.: Medical Physiology and Biophysics, Saunders: Philadelphia, 1961. p. 1016.
241. White, A., Handler, P. and Smith, E.L.: Principles of biochemistry. 3rd Ed. (1964): McGraw Hill.
242. Giese, A.C.: Cell Physiology. Saunders: Philadelphia, p. 195.
243. Handbook of Physiology - circulation, VII, Williams and Wilkins, Baltimore, p. 1061.
244. Handbook of Physiology - circulation, VII, Williams and Wilkins, Baltimore, p. 1059.
245. Handbook of Physiology - circulation, VII, Williams and Wilkins, Baltimore, p. 996.
246. Eisman, B.: Metabolism of basal motor agents by the isolated perfused lung. J. Thorac. Cardiovasc. Surg., 48: 798, 1964.
247. Cooper, T., Jellinek, M., Willman, V.L., Schweiss, J.F. and Hanlon, C.R.: Plasma histamine during cardio-pulmonary bypass in man. Arch. Surg. (Chicago), 88: 138, 1963.
248. Wegelius, O. and Von-Knorring, J.: Hydroxyproline and hexosamine content of human myocardium at different ages. Acta Med. Scand., 175: Supp. 412, 233, 1964.
249. Douglas, C.R., Jorquera, J.A., Pavez, P., Escobar, E. and Rosenkranz, A.: Mucopolysaccharides content of arterial wall in the rat upon emptying of mast cells. Acta. Physiol. L.A., 14: 37, 1964.
250. Ciricuta, I., Papilian, C., Rogozan, I.: Capillary permeability in burns. Roumanian Med. Rev., 7: 3, 1963.





251. Hawk, P.B., Oser, B.L. and Summerson, W.H.: Practical physiological chemistry. Toronto, McGraw-Hill, 1954, p.568.







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